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A SYNDROME OF EUNUCHOIDISM WITH SPERMATOGENESIS, NORMAL URINARY FSH AND LOW OR NORMAL ICSH: ("FERTILE EUNUCHS")*

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WHETHER androgen deficiency is of the severest grade, as in eunuchism, or of less severe grade, as in eunuchoidism, and whether it is of testicular or of hypophyseal origin, the testes are almost invariably aplastic or atrophic and in consequence distinctly small. To find normal-sized testes in an individual who otherwise has all the attributes of a eunuch is an impressive sight and is the key to the syndrome to be described. The finding of normal or nearly normal-sized testes under such circumstances indicates two simple facts: a) that eunuchism is not testicular failure, but androgen deficiency; and b) that testicular mass depends largely on the bulk of the tubules present.

Eunuchs and eunuchoids are almost invariably sterile and, so far as we can judge from our experience and that of others, there is seldom spermatogenesis to be demonstrated by testicular biopsy. In the 5 patients described here, there were moderate numbers of spermatozoa in almost all tubules prior to treatment; and in 2 there were normal numbers of sperm in the semen. Hurxthal *et al.* (1) reported 5 eunuchoid patients treated with testosterone and chorionic gonadotropin, who showed spermatogenesis after treatment. In 3 of his cases the absence of sperm in the semen before

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treatment was assumed, since no semen examinations were available. Testicular biopsies performed in 2 patients, 1 before and 1 after treatment, showed a small number of Leydig cells and few or no sperm in the tubules. Spermatogenesis following treatment occurred in 3 cases, as shown by semen examinations. Although such examinations were not obtainable in the 2 remaining patients, spermatogenesis was assumed to have occurred, since the wives became pregnant. Hurxthal's patients would not fall in the same category as the patients described here, provided the FSH excretion in them was actually deficient as the authors claimed.

So far as we know, in most extreme cases of eunuchoidism, actual fertility does not exist. However, in some patients with androgen deficiency of moderate grade, fertility may be present; that is, sperms may exist in the tubules, but there is little or no semen plasma to transport them. Although it is not scientifically correct, for the sake of emphasis we have called such patients "fertile eunuchs."

In the syndrome described here the urinary follicle-stimulating hormone (FSH) titer, which is related to the maintenance of relatively normal tubular structure, was found to be normal. In this respect it is to be contrasted with the high titers of urinary gonadotropin so frequently found in patients with tubular damage without any evidence of androgen deficiency.

The measurement of urinary interstitial-cell stimulating hormone (ICSH) was performed in an effort to shed some light upon the Leydig cell-pituitary imbalance which was anticipated. The limited experience acquired in the use of the rat prostatic phosphatase test for ICSH shows it to be roughly quantitative and apparently adequate for most clinical purposes. This test indicated that in 3 of our patients there was a deficiency of urinary ICSH, whereas in the remaining 2 it was considered to be present in normal amounts.

The Leydig cells, which in the normal testes stand out boldly in well defined islands, could be located in these testes with difficulty, with uncertainty, or not at all. Careful counts[1] of Leydig cells were performed on slides made from biopsy specimens of these testes. They showed that Leydig cells were either absent, or reduced decidedly below the normal range. The fact that there was severe functional deficiency of androgen production was clearly evident from the clinical signs and was corroborated by the levels of urinary 17-ketosteroids, which were comparable to those usually seen after castration. That a severe degree of androgen deficiency can exist in association with relatively normal tubular function is interesting.

[1] By Dr. J. B. Hazard, Department of Pathology, Cleveland Clinic Hospital.

In women, it is generally accepted that a failure of luteinizing-hormone (LH) production leads to failure of ovulation. A similar selective gonadotropin deficiency has been postulated to occur in men, but up to the present time patients demonstrating this abnormality have not been reported. We believe that 3 of the patients reported here, whose ICSH assays showed low values, represent instances of this syndrome; we assume that, in them, the Leydig cell failure was secondary to a selective deficiency of pituitary ICSH. To test the validity of this hypothesis we treated these patients with chorionic gonadotropin. In Case 3 the symptomatic response was striking—erections became frequent but semen was never produced; although we had hoped for decided improvement in the other cases, only moderate symptomatic changes were observed. We feel that it is to be anticipated that when Leydig cell deficiency is primarily testicular in origin and is sufficiently severe, ICSH titers will be elevated. However, the 2 patients in this series who did not have abnormally low ICSH levels, excreted amounts which, so far as we know, lie within normal range. In 1 of these patients the response to treatment with chorionic gonadotropin was striking, and the sperm count apparently increased. In the other patient there was little symptomatic change.

The salient characteristics of the syndrome here described are the presence of spermatogenesis despite moderate to severe androgen deficiency, whether this deficiency be of primary testicular origin or secondary to pituitary failure.

Although the extreme form of this syndrome is apparently rare, it is not likely that a clearer understanding of the principles involved may lead to the detection of many milder cases. It now seems apparent that infertility may sometimes be explained on this basis. Much greater experience will be required to determine the value of therapy in such cases.

METHODS

Urinary ICSH was assayed by the method of Schaffenburg and McCullagh (2), based on the specificity of response of prostatic alkaline phosphatase to purified ICSH[2] and androgen, in the hypophysectomized rat. Urinary extracts were prepared by ultrafiltration of 48-hour urine specimens and assayed in hypophysectomized, immature male rats, with the exception of extracts from 1 patient (A.S., Case 3), which were assayed in hypophysectomized adult rats. Each 48-hour extract was analyzed in 2 rats, each rat receiving the extract from a 24-hour portion of the urine specimen, dissolved in water and distributed in six injections given over a

[2] The preparation used was purified sheep pituitary ICSH obtained through the courtesy of Dr. R. K. Meyer, University of Wisconsin.

period of three days. Due to the variability of response from group to group, a standard composed of rats injected with known amounts of chorionic gonadotropin was run along with each assay. The rats were sacrified four days after the first injection, and the prostatic alkaline phosphatase concentration in tissue homogenates was determined by the method of Huggins and Talalay (3). Results are expressed as prostatic phosphatase content in Huggins-Talalay units, and are to be compared with those in untreated controls and chorionic gonadotropin-injected rats. In the case of the adult rats the aim was the prevention of the fall of phosphatase concentration after hypophysectomy. These rats were injected daily, beginning one day after hypophysectomy, with either urinary extracts or standard chorionic gonadotropin for eight days, and sacrified on the ninth day; results, expressed as described, were compared with those in untreated hypophysectomized controls.

The results obtained in the two 24-hour specimens were averaged, and represent the ICSH content of a 24-hour urine in a semiquantitative fashion. Twenty-four-hour urine extracts from 22 apparently normal men, ranging in age from 21 to 55 years, have shown values of 3.20 to 8.64 alkaline phosphatase units per rat prostate, with an average of 6.14 units —roughly equivalent to ± 250 γ of standard chorionic gonadotropin. Values significantly deviating from these seem to be definitely abnormal.

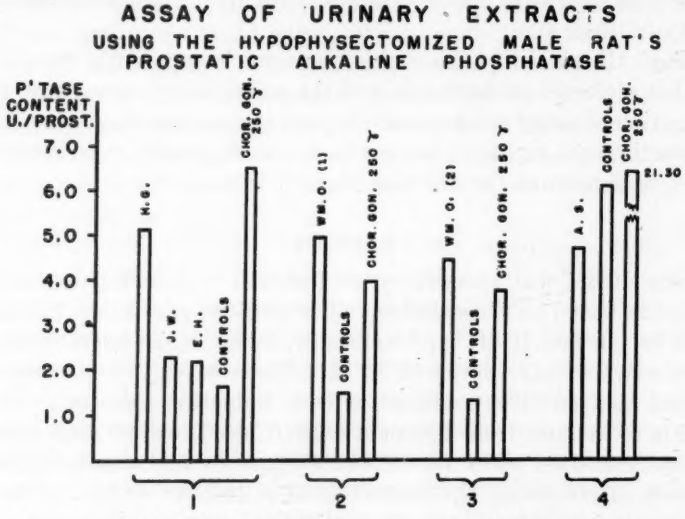

FIG. 1. Numbers 1 to 4 (below brackets) represent individual assays run at different times. Controls are values for uninjected, hypophysectomized rats. Rats injected with standard preparation received 250 gamma of chorionic gonadotropin. Columns bearing patients' initials represent average values for two 24-hour urinary extracts.

Results of assays in the 5 cases to be described are graphically presented in Figure 1.

Urinary FSH was assayed by the method of Klinefelter, Albright and Griswold (4), and 17-ketosteroids by the method of Bowman (5).

CASE REPORTS

Case 1 (E.H.).

A 39-year-old man came for examination because of failure of development of the genitalia, lack of beard growth, and a high pitched voice. Inquiry revealed that he had always been slightly obese, that there had been neither libido nor potency at any time and that an erection had rarely occurred. Endurance had always been poor and he had been able to do only light work. Past history was noncontributory except for mumps at the age of 10, with no accompanying orchitis. One brother had, on one brief examination, demonstrated all the attributes of a eunuch but would not permit complete clinical investigation.

FIG. 2. E. H., Case 1.

FIG. 3. E. H., Case 1.

Physical examination revealed an obese man weighing 206½ pounds (93.6 Kg.) with a height of 5 feet 4 inches (1.63 M.) (Figs. 2 and 3). The skin was dry and finely wrinkled, axillary and pubic hair were scanty (the latter being of female distribution) and there was no beard. The penis was exceptionally small, the scrotum well formed, and the

testes slightly soft, measuring 2.8 cm. by 1.5 cm. The prostate was one-third average normal size.

This patient was not seen again until eleven years later, when he reappeared with essentially the same history and physical findings. In the interim there had been an occasional erection and slight sex desire. Testicular size at this time was 3.5 cm. by 2.5 cm.

Laboratory examinations, including urinalysis, Wassermann and Kahn reactions, and a complete hemogram revealed no abnormality. The B.M.R. was −6 per cent. X-ray examination showed the sella turcica to be normal. Roentgenograms of the left wrist indicated skeletal maturity. Urinary gonadotropins were more than 26 and less than

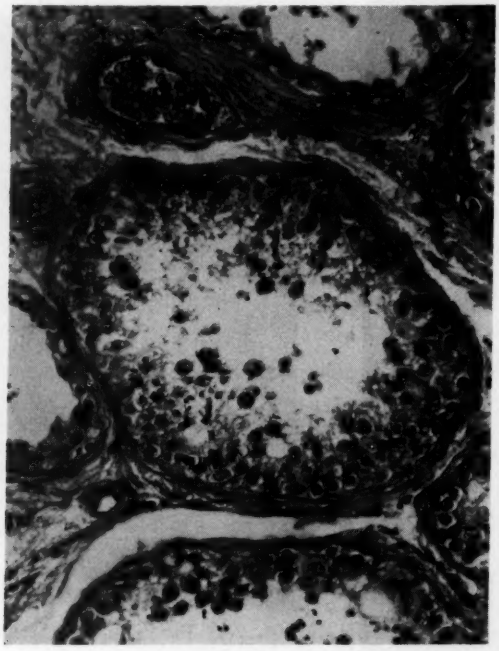

FIG. 4. Histologic section from testicular biopsy specimen in E. H., Case 1(×200). For explanation *see* text.

52 m.u. per twenty-four hours on two occasions. Urinary ICSH was definitely below normal (E. H., Fig. 1) and 17-ketosteroids were 4.9 and 4.7 mg. per twenty-four hours. Capon comb-growth assay for androgen (using two birds for each 24-hour specimen) showed comb growths of 2 mm., 2 mm., and 0 mm., 0 mm. These figures represent total comb growth—length plus height. Specimens of urine from normal men, extracted in the same manner, give comb growths varying usually between 8 and 18 mm. (6).

Testicular biopsy (Fig. 4) revealed that spermatogenesis, though less than normal in activity, was present; spermatozoa were seen in almost every tubule. There was some thickening of the basement membrane of the tubules. The interstitial tissue was of moderately increased density and only a rare interstitial cell was found; the average for both

testes was a maximum of 0.5 Leydig cell per tubule, as compared to a normal of 7 Leydig cells per tubule for a 39-year-old man (7).

It was not possible to carry out semen examinations until three years later—after testosterone therapy which, because of poor cooperation, had unfortunately been intermittent and inadequate. The semen then contained an occasional sperm. The patient was given testosterone and noted growth of the genitalia, erections and nocturnal emissions. He also began to masturbate.

This patient has been re-examined recently. For most of the preceding three years he had taken 10 mg. of testosterone by buccal absorption on alternate days, and 50 mg. of testosterone propionate intramuscularly weekly. He still looked distinctly eunuchoid, had no beard and sparse body hair, although there were increased amounts of axillary and pubic hair; the penis and prostate were about half average normal size. Sexual stimulation was moderate and his energy and endurance were greatly increased. For example, during the two preceding autumns he had been able to do strenuous harvest work without undue fatigue, an experience he had never had before. He was still obese. His blood pressure was 160/80, B.M.R. −1 per cent, and blood cholesterol level 217 mg. per 100 cc. Semen examination after one day of continence showed: volume, 5.1 cc.; concentration of sperm, 8,000,000 per cc.; and total number, 40,800,000. After one hour, an occasional sperm showed a normal degree of activity. A more recent semen examination showed: volume, 5.0 cc.; concentration of sperm, 4,000,000 per cc.; total number 20,000,000. After one hour an occasional sperm showed 4 plus motility. Treatment was changed to chorionic gonadotropin,[3] 4,000 International Units (I.U.) weekly. In the patient's own estimation, the effects on libido, potentia and general well being were not as extensive as those obtained after testosterone therapy. Objective evaluation of this form of treatment has not been possible as yet.

Case 2 (J.W.).

A 40-year-old man was referred to us by the surgeons because of a eunuchoid appearance and gynecomastia. He had gone to them because of right upper quadrant pain. He gave no history of mumps or of orchitis. He could not remember at what age puberty occurred. The gynecomastia, he believed, had increased slightly since the age of 20. He married at the age of 21 and when he was 29 his wife gave birth to a daughter, their only child.

He had never shaved more than once a week and stated that he really did not need to shave that often, as there was only a slight beard growth in the lateral chin and lateral moustache areas. Sex drive had always been weak; desire for coitus was noted about once every two or three months. Energy had always been at a low level; even as a young man, after an ordinary day's work he felt "all worn out" and was usually tired in the morning.

Physical examination. He was a small (5 feet 4 inches, or 1.63 M.), pale, mild-appearing, immature person (Figs. 5 and 6). There was no recession of the temporal hairline, almost no apparent beard, almost no hair on the chest or abdomen, very sparse hair on the lower legs and forearms, and very sparse axillary and pubic hair. Gynecomastia was diffuse and of moderate degree. The penis was distinctly smaller than the average normal size. The testes were of small-normal size and slightly soft. The prostate was considered to be normal in size. At the time of the first examination we found it almost impossible to believe that he was a father.

[3] APL (gonadotropic hormone from human pregnancy urine)—Ayerst, McKenna and Harrison, Ltd.

Laboratory examinations, including urinalysis, white blood count, hemoglobin, Wassermann and Kahn reactions, and a blood sugar test, gave normal results. A cholecystogram revealed multiple, non-opaque calculi (cholecystectomy was eventually performed).

The remainder of the laboratory findings include a semen examination: volume, 2.1 cc.; concentration of sperm, 102,000,000 per cc.; and total number, 214,000,000. Motility was reduced, only 15 per cent being highly motile; 11 per cent showed abnormal forms.

FIG. 5. J. W., Case 2.

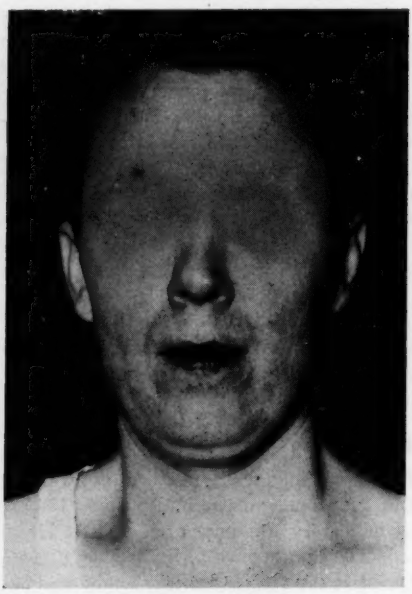

FIG. 6. J. W., Case 2.

This specimen of semen was brought from home and because we were still incredulous, the patient was placed in a hospital room and requested to produce a specimen of semen by masturbation. The report was as follows: volume, 3.1 cc.; concentration of sperm, 81,000,000 per cc.; total number 251,000,000; motility, 4 plus in 25 per cent of the spermatozoa present; 11 per cent abnormal forms.

Urinary gonadotropins (FSH) assayed more than 52 and less than 105 m.u. per twenty-four hours. Urinary 17-ketosteroids were 5.9 and 6.9 mg. per twenty-four hours.

Testicular biopsy (Fig. 7) showed active spermatogenesis. The tubules were of average size; spermatogonia showed many mitoses and all stages of spermatogenesis were seen, with fairly numerous spermatids and spermatozoa. The basement membranes were of average thickness. The only abnormality noted was the complete absence of Leydig cells in the sections examined.

Recent examination of this patient, five years after he was first seen, showed his appearance and physical findings substantially unchanged. He had received no treatment and it was not until the situation had been discussed at length that he showed some interest in it. Semen examination was as follows: volume, 2.6 cc.; concentration of sperm, 40,000,000 per cc.; and total number, 104,000,000. Two hours after ejaculation, only a few sperm showed 3 plus to 4 plus motility; seven hours after, only a rare sperm showed 4 plus motility.

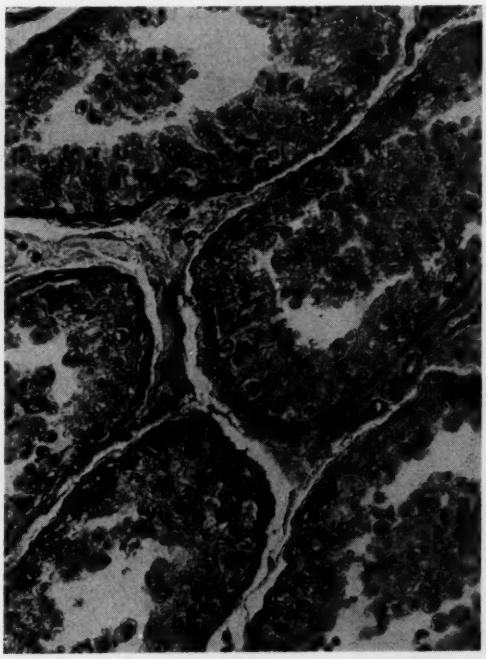

FIG. 7. Histologic section from testicular biopsy specimen in J. W., Case 2 (×200). For explanation *see* text.

An assay of urinary ICSH showed the hormone content of two 24-hour specimens to produce a stimulation of the rat's prostatic phosphatase which was barely above that produced by untreated controls (J. W., Fig. 1).

Treatment was begun with chorionic gonadotropin,[3] 2,000 I.U. weekly, the dose being gradually increased to 4,000 I.U. weekly. After four weeks of such therapy, the patient reported a slight increase in energy and libido. Urinary 17-ketosteroids were 3.1 mg. per twenty-four hours. Intercurrent illness prevented further investigation.

Case 3 (A.S.).

A 21-year-old man came for advice because his penis was abnormally small. His past history revealed an uneventful delivery with normal early development. Masturbation had been practiced, but ejaculation had never occurred. Voice change had apparently

begun at about the age of 15 years, but had never been complete, and he had never shaved. He first became aware that his genitalia were smaller than average at the age of 15, but this did not become a real concern to him until he was 18, at which time he sought medical aid. A "pituitary gland injection" was given every two weeks for a period of four to six months, and one year later a similar course was administered, lasting two months; both were without subjective or objective effect.

Family history was noncontributory, except for the fact that his father was slow in development and did not begin shaving until he was 21 years old. His only brother was married and had two children.

FIG. 8. A. S., Case 3.

FIG. 9. A. S., Case 3.

Physical examination revealed a well nourished individual (Figs. 8 and 9), 5 feet 7¼ inches (1.71 M.) tall, weighing 164 pounds (74 Kg.), with a low frontal and temporal hairline and no facial hair. The amount of axillary and pubic hair was less than normal, the latter having a gynecoid distribution. The hair on the forearms and legs was normal. The penis was small, measuring 5 cm. in length. The testes were slightly reduced in size, the left measuring 4 cm. by 2 cm. and the right being slightly larger; they were of normal shape and consistency. The scrotum was rugose, and the prostate was of childhood type. The clinical diagnosis was eunuchoidism of moderate degree.

Laboratory examinations, including urinalysis, white blood count, hemoglobin, Wassermann and Kahn reactions and a blood sugar test, gave normal results. The B.M.R. was

−21 per cent. Roentgenograms showed the chest and sella turcica to be normal. Bone age, according to the Todd atlas, was placed at 15 years 9 months. Urinary gonadotropins (FSH) were more than 6 and less than 105 m.u., and urinary 17-ketosteroids were 6.1 mg. per twenty-four hours.

Sexual intercourse had been attempted on several occasions and was considered by the patient to be normal in all respects, except that there was no suggestion of an ejaculate. For similar reasons no semen specimen could be produced by masturbation.

Urinary ICSH was assayed in hypophysectomized adult male rats; there were no measurable quantities in two 24-hour specimens (A. S., Fig. 1).

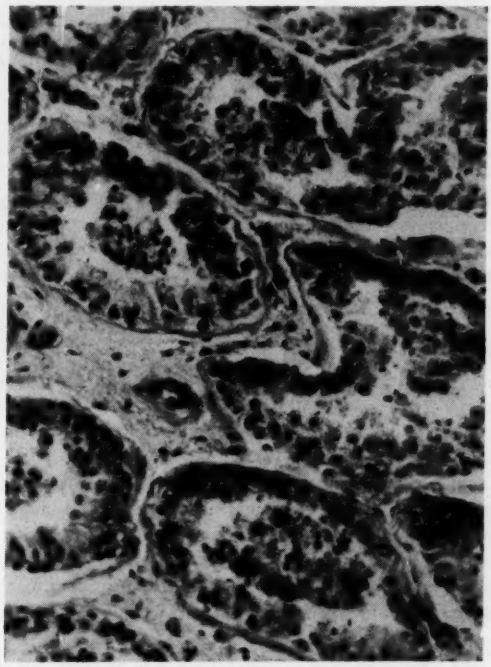

FIG. 10. Histologic section from testicular biopsy specimen in A. S., Case 3 (×200). For explanation *see* text.

The diagnosis of eunuchoidism was based on his beardlessness, high-pitched voice, skeletal immaturity, small penis, infantile prostate and lack of normal prostatic and vesicular secretions, as evidenced by absence of semen.

Testicular biopsy (Fig. 10) showed active gametogenesis with spermatozoa but no recognizable Leydig cells. Therapy was begun with chorionic gonadotropin[4] 2,000 I.U. intramuscularly twice weekly. When seen one month later, the patient stated erections were frequent, occurring from two to three times per day as compared with twice a month previously. The testes and penis seemed larger and acne had appeared on his

[4] Pranturon (gonadotropic hormone from human pregnancy urine)—Schering Corporation.

chin. It seemed evident to the observers that there had been an appreciable increase in androgen production as the result of treatment.

Case 4. (H. G.).

A 21-year-old man was referred for study because there was a suspicion of hypopituitarism or hypogonadism. The only complaints at this time were weakness and easy fatigability.

Past history appeared to be irrelevant. At the age of 14 years delay in puberty had been suspected. In the intervening period he continued to be tall and thin but failed to reach a point of masculine maturity which his physician deemed to fall within the range of normal. He had shaved infrequently. Family history was noncontributory.

Physical examination revealed a tall, slender young man whose height was 6 feet 3 inches (1.91 M.) and weight 150 pounds (68.1 Kg.). A photograph of this patient could not be obtained. He was almost beardless, and there was distinctly less than an average amount of body and sexual hair. The penis was smaller in size than normal, there was a slight varicocele on the left side and the testes were of normal size and consistency. Unfortunately, no specific observation of prostatic size was made on the initial examination. The clinical impression was hypogonadism of mild degree.

Laboratory examinations, including urinalysis, hemoglobin, white blood count and differential, a blood sugar test, and Wassermann and Kahn tests, gave normal results. Urinary gonadotropins were more than 13 and less than 105 m.u. per twenty-four hours. Urinary 17-ketosteroids were 7.9 mg. per twenty-four hours. Semen examination showed: volume, 5.2 cc.; concentration of sperm, 600,000 per cc., and total number, 3,120,000; 65 per cent showed 4 plus motility at the end of one hour.

Testicular biopsy (Fig. 11) demonstrated fairly active gametogenesis with relative rarity of recognizable Leydig cells; those present were distinctly aplastic and actual counts revealed a number of up to 0.6 Leydig cell per tubule (normal value, 9 Leydig cells per tubule for a 21-year-old man (7)).

Therapy was begun with 750 I.U. of chorionic gonadotropin[3] three times weekly. There was a distinct subjective improvement in strength and energy when the patient was seen three months later. Daily shaving had become a necessity and erections and nocturnal emissions were more frequent. Hair growth on the forearms, legs, and abdomen had increased. Therapy was continued at a lower dosage (500 I.U. three times weekly) with continued improvement in strength, a 15-pound weight gain in one year, an increase in the size of the musculature, and an increase in the sperm count.

At the end of one year chorionic gonadotropin was stopped for a six-month period, with a resultant decrease in strength and endurance. This patient's libido fluctuated with his vigor. Recently the dosage of gonadotropin was increased to 5,000 I.U. weekly and the beard growth became even more rapid. After thirteen weeks of such therapy the urinary 17-ketosteroids were unelevated, being 8.0 mg. per twenty-four hours, and the urinary FSH level was between 13 and 105 m.u. per twenty-four hours. Urinary ICSH levels in two 24-hour specimens were well within the normal range (H. G. Fig. 1).

Serial semen examinations are presented in Table 1 and it will be seen that progressive mprovement in the sperm count occurred, the maximum level being reached sixteen weeks after cessation of a one-year course of injections of chorionic gonadotropin. This suggests that there had been a partial inhibition of spermatogenesis, similar to that seen after testosterone administration, and that a rebound followed the withdrawal of the chorionic gonadotropin. It is also of interest that, while the patient was receiving 250 I.U. of chorionic gonadotropin per week, the sperm count fell to unusually low levels, suggesting that this dosage level was inadequate.

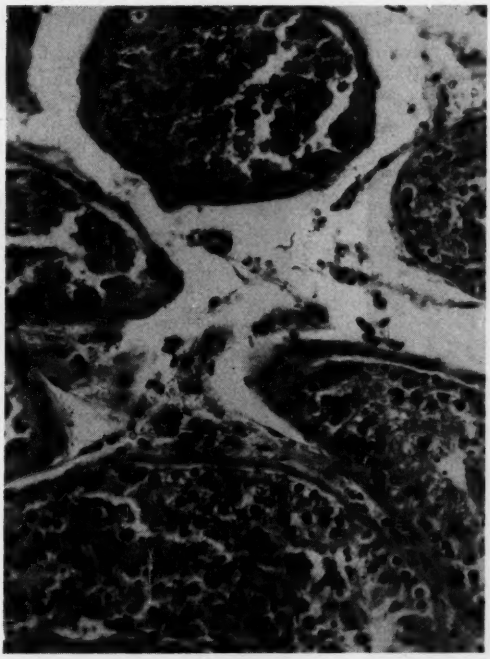

FIG. 11. Histologic section from testicular biopsy specimen in H. G., Case 4 (×200). For explanation *see* text.

TABLE 1. SEMEN EXAMINATIONS IN CASE 4

Date	Vol. (cc.)	No. of sperm per cc.	Total No. of sperm	Motility (1 hr.)	Morphology	
Apr. 25, '49	5.2	600,000	3,120,000	65 per cent, 4 plus		*Before treatment*
Oct. 29, '49	5.0	200,000	1,000,000	Too few to estimate		750 I.U. chor. gonadotropin 3 times per week for 16 wks.
Feb. 18, '50	5.9	5,000,000	29,500,000	Occasional, 4 plus	Too few to count	500 I.U. chor. gonadotropin 3 times per week for 16 wks.
July 8, '50	6.8	500,000	3,400,000	Occasional, 4 plus	Too few to count	500 I.U. chor. gonadotropin 3 times per week for 20 wks.
Oct. 30, '50	5.6	14,000,000	78,400,000	25 per cent, 4 plus	23 per cent abnormal forms	No therapy for 16 wks.
Mar. 3, '51	5.6	10,000,000	56,000,000	35 per cent, 4 plus		250 I.U. chor. gonadotropin 3 times per week for 11 wks.
Sept. 4, '51	5.1	Too few to count				250 I.U. chor. gonadotropin for 4 wks. after having none for 2 mos.
Oct. 8, '51	6.8	4,000,000	27,200,000	50 per cent, 3 plus		5000 I.U. chor. gonadotropin per wk. for 5 wks.
Feb. 25, '52	1.7	6,000,000	10,200,000	Occasional, 3 plus		Last injection 15 days before.

Case 5. (Wm. O.).

This patient was 23 years of age when first seen and had come for a general examination. He stated that puberty had been somewhat retarded and that at the age of 15 he had been given "pituitary" injections to stimulate growth. Past and family histories were noncontributory.

FIG. 12. Wm. O., Case 5.

FIG. 13. Wm. O., Case 5.

Physical examination revealed a white man measuring 5 feet 11½ inches in height (1.81 M.) and weighing 168 pounds (76 Kg.) (Figs. 12 and 13). His facial appearance was boyish. The abdomen was obese. The penis was smaller than average, but the testes were normal in size and consistency. The prostate was considered to be within the lower limit of normal in size. Pubic and axillary hair were normal and there was a fair amount of hair on the legs. The beard was sparse and there was no beard on the cheeks.

Laboratory examinations, including blood count and hemoglobin, a blood sugar test. Kahn and Wassermann tests and urinalysis, gave normal results. The B.M.R. was −15 per cent. Urinary gonadotropins were more than 13 and less than 26 m.u., and 17-ketosteroids 8.3 mg. per twenty-four hours.

Semen examinations showed: volume, 3.3 cc.; concentration of sperm, 27,000,000 per cc.; and total number, 89,000,000. At forty-five minutes 50 per cent of the sperm, and at three hours 20 per cent, showed a motility of 4 plus.

It was decided this patient represented a mild case of adiposogenital dystrophy,

and a weight reduction diet was instituted. Semen examination one month later revealed: volume, 4.2 cc.; concentration of sperm 46,000,000 per cc.; and total number, 193,000,000 sperm. At one hour and forty-five minutes, 5 per cent of the sperm showed a motility of 3 plus. Three months later the semen volume was 3.0 cc.; concentration, 51,000,000 per cc.; and total number, 153,000,000. At the fourth hour, 30 per cent of the sperm showed a motility of 4 plus. The patient had lost 8 pounds in weight.

This patient was not seen again until six years after his initial visit, when he was reexamined. He had been married for two years and his union had been childless. He came expressing his desire to have children, the lack of children being his only complaint.

Physical examination disclosed substantially the same findings as previously. Weight had increased to 175¾ pounds (79 Kg.). A urinary FSH assay showed levels of 13 to 105 m.u. per twenty-four hours. Urinary ICSH assay on two occasions showed the 24-hour levels to be normal (equivalent to about 250 I.U. of chorionic gonadotropin; *see* Fig. 1, Wm. O., No. 2 and 3). Semen examinations showed: volume, 4.4 cc.; concentration of sperm, 21,000,000 per cc.; and total number, 92,400,000. At one hour, 33 per cent of the sperm, and at four hours 23 per cent, showed a motility of 3 plus.

Testicular biopsy. Because the general findings in this case were suggestive of the syndrome of eunuchoidism with spermatogenesis, a testicular biopsy was performed. The report was as follows: most tubules are of average size and show normal degrees of spermatogenesis (Fig. 14); there are a few small tubules with marked thickening of the base-

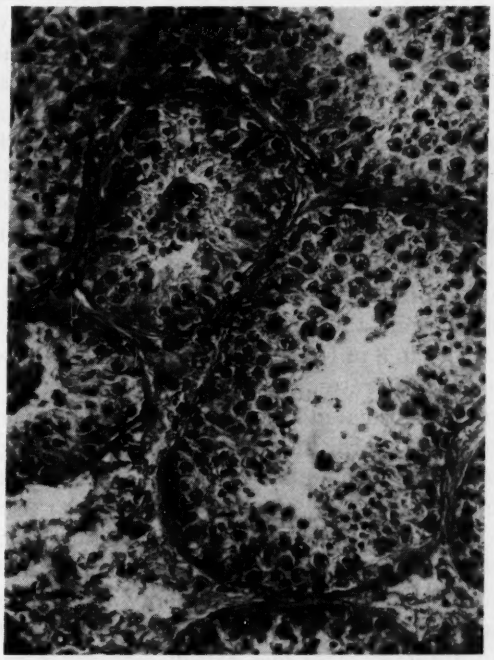

FIG. 14. Histologic section from testicular biopsy specimen in Wm. O., Case 5. (×200). For explanation *see* text.

ment membrane and only a few cells in the lumen. The interstitial tissue is of average density except for a few areas where it is increased. Leydig cells are few and scattered and no crystalloids are evident as shown by both Masson and H. and E. stains. Actual count of these cells shows an average of 0.5 Leydig cell per tubule as compared to an normal average of 9 Leydig cells per tubule for an individual of that age (7).

Treatment was started with 2,000 I.U. weekly of chorionic gonadotropin.[3] After nine weeks of such therapy a semen examination showed: volume, 5.1 cc.; concentration of sperm, 12,000,000 per cc.; and total number, 61,200,000. At one hour, 8 per cent of the sperm showed a motility of 2 plus. Because the benefit from this treatment was not very striking, the patient was given testosterone cyclopentylpropionate, 200 mg. a week.

DISCUSSION

A summary of the pertinent findings in these cases is presented in Table 2. All the patients had a eunuchoid habitus, which varied only in the degree of severity. In 3 cases, there was scant axillary and pubic hair with a female distribution of the latter. In 2, beard growth was completely absent, and in the others, it was exceptionally slight. Only 1 patient displayed gynecomastia. The external genitalia, except for the testes, were infantile in 1, decidedly small in 2, and only slightly reduced in size in the remaining 2. The testes were slightly reduced in size in 1 case and normal in the rest. This finding is in keeping with the normal complement of tubular elements seen in the biopsy sections, the tubules making up the larger part of the testicular mass, and the interstitial cells contributing a much smaller portion. The prostate was small in 2 patients; alteration in size and consistency was not noted on the initial examination in the other three.

X-ray films showed the sella to be normal in all instances. An estimate of skeletal maturity was made in 2 cases. In the 21-year-old patient (Case 3), the bone age was 15 years and 9 months, as estimated by the Todd atlas; this fact is further evidence that, in this person, maturity was materially retarded. In the 39-year-old patient (Case 1) all epiphyseal lines were closed, despite the clinical appearance of eunuchism. The low B.M.R. values found in 3 of these patients are consistent with those commonly found in male castrates.

The semen was not normal in any of these patients. Semen examinations were impossible before treatment in 2 instances (Cases 1 and 3). Evidently, in the most severely eunuchoid patient (Case 1), there was not sufficient circulating androgen to produce prostatic and vesicular secretions in quantities adequate to provide the necessary fluid medium to carry the sperm known to be present in the tubules. After some years of androgen therapy, which had been sufficient to increase his energy and general vigor greatly, though not enough to overcome completely the signs of eunuchism, a semen specimen obtained after one day of continence showed a normal volume with a count of 8,000,000 per cc. In Case 3 semen had never been

TABLE 2. SUMMARY OF FINDINGS IN 5 CASES OF EUNUCHOIDISM WITH SPERMATOGENESIS

Patient	Case 1 E.H.	Case 2 J.W.	Case 3 A.S.	Case 4 H.G.	Case 5 Wm.O.
Age (yrs.)	39	40	21	21	23
External genitalia	Small penis. Testes ? small	Small penis. Testes, moderate size	Small penis. Testes, moderate size	Small penis. Testes, normal size	Small penis. Testes, normal size
Hair distribution and type	Female	Female	Female	Relatively beardless. Otherwise normal	Sparse beard. Otherwise normal
Clinical impression	Eunuchoidism, severe	Eunuchoidism, definite	Eunuchoidism, moderate	Eunuchoidism, mild	Eunuchoidism, mild
B.M.R., %	−6	Not obtained	−21	−13	−15
Sella turcica by x-ray exam.	Normal	Normal	Normal	Normal	Normal
Semen examination	On Jan. 6, 1949, occasional sperm (13 years after initial visit.) After 3 years of inadequate testosterone therapy, 8,000,000/cc.; 40,800,000 total; occasional 4 plus motility	Sperm, 102,000,000/cc.; 214,200,000 total; 15 per cent showed 4 plus motility	None obtainable	Sperm, 600,000/cc.; 3,120,000 total; 65 per cent showed 4 plus motility	Sperm, 27,000,000/cc.; 89,000,000 total; 50 per cent showed 4 plus motility
Urinary gonadotropins (FSH)	>26 <52 m.u./24 hrs.	>52 <105 m.u./24 hrs.	>6 <105 m.u./24 hrs.	>13 <105 m.u./24 hrs.	>13 <26 m.u./24 hrs.
Urinary ICSH	Low	Low	Low	Low, normal	Normal
Urinary 17-ketosteroids	4.9 4.7 mg./24 hrs.	5.9 6.9 mg./24 hrs.	6.1 mg./24 hrs.	7.9 mg./24 hrs.	8.3 mg./24 hrs.
Testicular biopsy	Active gametogenesis. Rare or absent Leydig cells	Active gametogenesis. Hypoplasia of Leydig cells	Active gametogenesis. No Leydig cells	Active gametogenesis. Hypoplasia of Leydig cells	Active gametogenesis. Hypoplasia of Leydig cells
Leydig-cell count per tubule	0.5	0	0	0.6	0.5

produced. In Case 2, a father who was moderately eunuchoid, although the sperm count reached the normal range, the volume of semen was at or about the low normal limit and the percentage of highly motile sperm was very low. Although this man was evidently fertile, his semen could not be described as normal.

In Case 4 the patient was, in our opinion, distinctly eunuchoid, but less severely so than any of the other 4 patients. Apparently, his level of circulating androgens had not been sufficient to bring about fully developed secondary sex characteristics, but it was sufficient for a normal volume of semen and for a degree of sperm motility which, though not normal, was not severely impaired. The normal semen volume and the ICSH levels did not fully support the diagnosis of Leydig-cell deficiency, but the clinical eunuchoidism and very low Leydig-cell count led us to include him as an example of a mild case of this syndrome.

Urinary gonadotropins (FSH) were normal in all instances. It is generally accepted that eunuchoidism may be associated with either abnormally low urinary gonadotropin titers, as in patients with pituitary failure, or with abnormally high titers, if severe primary damage has occurred to the testes. It is also well established that gametogenic failure without any evidence of androgen deficiency is usually associated with high titers of urinary FSH. The fact that urinary gonadotropins in the eunuchoid men described previously were normal, is impressive. It should be kept clearly in mind that urinary gonadotropins (usually measured as, and frequently called FSH) are in reality a mixture of gonadotropins in which the apparent amount of FSH present may vary considerably according to the concentration of LH (ICSH), and perhaps also according to the concentration of luteotropin (prolactin). The converse, however, is not true; that is to say, in the measurement of ICSH by the phosphatase method there is no apparent increase in ICSH after the addition of large amounts of FSH or luteotropin (2). In our series, the measurement of urinary ICSH by a semiquantitative method indicated that low values were present in 3 of the patients, whereas they were normal in the remaining 2 patients.

Urinary 17-ketosteroids were at the lower limits of normal or below normal. In considering these figures it should be kept in mind that these determinations were made by a method (5) which usually yields lower values than are obtained with other methods. Normal levels for men, as determined in our laboratory, lie between 7 and 15 mg. per twenty-four hours. The patients reported here excreted 4.9 and 4.7, 5.9 and 6.9, 6.1, 7.9, and 8.3 mg. per twenty-four hours for Cases 1 to 5, respectively. These figures, though generally acknowledged as not diagnostic of hypogonadism, nevertheless are consistent with that diagnosis.

Testicular biopsy showed active gametogenesis in all cases with hypoplasia of the Leydig cells of varying degree, being considered most severe in Cases 2 and 3. The basement membranes were not decidedly abnormal. There was some doubt as to the degree of rarity of hypoplasia of the Leydig cells. Leydig-cell hyperplasia is a term frequently used and usually there is no proof that such a condition is more than a comparative superabundance of Leydig cells in a pattern of scanty tubular structures. In cases in which careful counts of Leydig cells have been made, when hyperplasia seems apparent in association with shrunken tubules, the proportion of Leydig cells to tubules is still normal (7). Leydig-cell hypoplasia seems easier to determine, but certainly not without some difficulties. Immature Leydig cells, such as those sometimes seen in pituitary failure, have more the appearance of a fibroblast than an active interstitial cell. If our judgment as to the response of these patients to chorionic gonadotropin is correct, then they must have had some immature or unstimulated Leydig cells and Leydig-cell precursors. In other words, rather than stating that Leydig cells were rare or absent, it would be probably more accurate to say that, by the histologic technics used, we saw few or no cells which appeared to us to be active and mature. In an attempt at quantitative measurement, we tried to count the Leydig cells present as accurately as possible with the technics available to us.

As to the actual site of failure of function, two possibilities are of interest. The first, as mentioned in the introduction, is a selective failure of ICSH production; the other, a primary failure of Leydig cells. Both have been postulated to exist but neither has been described to date. The former hypothesis is probably correct in 3 of our cases, as suggested by the absence of ICSH in the urine by the methods used. A primary Leydig-cell failure, must be presumed to exist in Cases 4 and 5; in these cases ICSH values were normal. The response to chorionic gonadotropin therapy appeared to be present in all, and especially manifest in Cases 3 and 4. The response in these patients was very striking and left no question in the mind of the clinicians that it could be due to anything but hormonal effects. It was slower in appearance than the response of severely eunuchoid men to testosterone, coming in several weeks rather than in several days. In intensity, however, it could be compared to the response expected from about 50 mg. of testosterone propionate three times weekly. There was a definite increase in vigor and strength, accompanied by an increase in libido, in the number of erections and nocturnal emissions. An increase in sperm count followed therapy in one instance. Beard growth became normal, and hair growth on the forearms, legs, chest and abdomen increased, as did the axillary and pubic hair.

A third possibility as to the mechanism involved, might be a neutraliza-

tion of endogenous ICSH before it reaches its target organ. This seems a much less likely explanation, because of the apparent response to injected chorionic gonadotropin.

SUMMARY

A syndrome of eunuchoidism with spermatogenesis occurring in 5 patients, ranging in age from 21 to 40 years, is described. The degree of eunuchoidism varied from mild to severe. The external genitalia and prostate were small in 4 cases and infantile in 1; the testes were of normal size in 4 and small in 1.

Total gonadotropins (FSH) were normal in all 5 cases and urinary ICSH, assayed by a semi-quantitative method, was low in 3 and normal in 2 cases. Semen examinations before treatment showed sperm counts ranging from 600,000 to 102,000,000 per cc. in 3 patients; after one hour, 15 to 65 per cent of the sperm showed 4 plus motility. In 1 patient who was severely eunuchoid, on initial examination, the sperm were too few to count; and in another, no semen was obtainable at any time. One patient was the father of a normal child. In 2, sperm counts were elevated after treatment with chorionic gonadotropin or testosterone.

Testicular biopsies showed active gametogenesis with spermiogenesis in all cases, with absent or hypoplastic Leydig cells. Urinary 17-ketosteroids were abnormally low or in a low normal range.

The response to chorionic gonadotropin was satisfactory in 3 cases and striking in 2.

Attention is called to the possible existence of milder forms of this syndrome, of which the main characteristics are the presence of spermatogenesis despite distinct clinical and laboratory signs of eunuchoidism.

Addendum

After this paper had been submitted, a Letter to the Editor of the *Journal of Clinical Endocrinology and Metabolism* (**13**: 128, 1953) from Dr. R. Q. Pasqualini came to our attention. Dr. Pasqualini presented a brief summary of a case (Pasqualini, R. Q., and Bur, C. E.: Sindrome hipoandrogénico con gametogénesis conservada, *Rev. Asoc. méd. argent.* **64**: 6, 1950), evidently similar to the 5 cases described by us. The hypothesis offered by Pasqualini as to the possibility of a deficiency in ICSH as a cause of the Leydig cell hypoplasia observed in these patients has been confirmed by us in 3 of the cases studied.

Acknowledgment

The technical assistance of Miss D. Tweed in the hormone assays is gratefully acknowledged.

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HYPOGONADISM WITH SPERMATOGENESIS: A CASE REPORT*

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TESTICULAR defects may conceivably involve both gametogenic and internal secretory functions, or either one of these processes with a relative or complete sparing of the other. Syndromes in which each of these possibilities apply have been described. The classic eunuchoid exhibits a gross hypogonadism with absent or arrested spermatogenesis. The group of patients first described by Klinefelter *et al.* (1) with hyalinization of the seminiferous epithelium, gynecomastia and high urinary gonadotropin titers, may have normal masculine secondary sex characteristics or exhibit signs of only a moderate androgen deficiency; and spermatogenesis may be seriously impaired without a suggestion of hypogonadism, as in most of the male sterility problems which come under observation. The third possibility, a Leydig-cell deficiency with little or no disturbance in spermatogenesis, has been described in a few patients with demonstrable lesions in the pituitary area (2), and McCullagh and Beck (3) have recently noted 4 patients with eunuchoidism and spermatogenesis with normal urinary FSH excretion. The patient studied in the present report undoubtedly fits into this latter very unusual group.

The lack of a specific sensitive index of testicular androgen output has made it almost impossible to substantiate the clinical impression of a partial or occult hypogonadism in a mature man. It is the absence of such a procedure which has led to manifest uncertainties concerning the so-called "male climacteric" (4), a disorder which as presently defined may be similar to the syndrome to be described. The case is one in which a mild androgen deficiency was suggested by a slight and perhaps questionable retardation in the development of the secondary sex characteristics, but strongly supported by an extended period of observation during which the semen fructose concentration was utilized as an objective physiologic indicator of androgen activity (5).

In man the fructose of semen is derived exclusively from the secretion of the seminal vesicles (6). The fructose content of these glands, which re-

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quire androgen for their development and maintenance, has been made the basis for a highly sensitive hormone indicator test in experimental animals (7). It was recently shown (5) that the fructose concentration of semen similarly responds to shifts in available male hormone, thus providing an androgen indicator for use in humans. The normal seminal fructose concentration in man was found to vary over a wide range (200 to 800 mg. per 100 ml.) but concentrations lower than 135 mg. per 100 ml., and especially below 100 mg. per 100 ml., were found only in untreated eunuchoids. The statistically significant correlation between androgen dosage and the resulting rise in semen fructose concentration in some of these eunuchoids suggested that serial estimations of seminal fructose in a suitable individual might well reflect prolonged alterations in his level of circulating androgen. It is this general procedure which has been employed here to establish the diagnosis of hypogonadism.

CASE REPORT

The patient, L. K., (Unit No. 293419) was 28 years old when seen for the first time in 1942. He was concerned because he had never developed a deep masculine voice, had a rather small phallus and very little beard growth. He shaved only once or twice a week. In spite of these signs of hypogonadism, he was interested in women, had nocturnal emissions and felt well. The only possibly pertinent point in his past history was that his right testis had been uneventfully brought down into the scrotum at the time of the repair of an inguinal hernia when he was 10 years old. No history of headaches or visual disturbances could be obtained.

On physical examination he was a healthy appearing young man of normal body proportions (Fig. 1). His skin was soft, with the faint yellowish pallor often seen in hypogonadism. Only a small amount of rather fine facial hair was noted. Pubic hair was abundant, axillary hair moderate in amount, and limb hair scant. His voice was somewhat high pitched, lacking resonating qualities. The laryngeal prominence was readily palpable, although perhaps slightly smaller than the average. The phallus was slightly undersized; the testes were also a little small but were firm in consistency. The right one measured 3.75 cm. by 2.5 cm.; the left, 4.0 cm. by 2.0 cm. Results of routine blood counts and urinalyses were normal. The Kahn test gave a negative reaction. X-ray examination of the skull disclosed a normal sella turcica. A semen specimen of only a few drops was obtained by masturbation. It contained many apparently normal spermatozoa. No treatment was begun at this time, as he entered the U. S. Army, having passed the induction physical examination without question.

Four years later he returned to the clinic, his general condition unchanged. Except for an injury resulting in partial deafness, his Army career had been uneventful. A semen specimen obtained at this time (March 10, 1947) contained 64,000,000 spermatozoa per ml., but the volume of the ejaculate was only 0.2 ml. The motility and morphology of the spermatozoa in the specimen were within normal limits. The fructose concentration was 33 mg. per 100 ml. as measured by the microglucose procedure of Miller and Van Slyke (8). All the results secured by this technique have been calculated as the reducing equivalent of glucose, but termed fructose, since Mann (6) has shown that essentially all of the reducing carbohydrate of human semen is fructose. This and subsequent ejaculates were prepared for the estimation of reducing carbohydrates within one hour of

emission. The patient was instructed to abstain for at least four days before producing any ejaculates for examination. Urinary 17-ketosteroids were 18.0 mg. per twenty-four hours, a normal value for the procedure used (9). A urinary gonadotropin assay, performed by Dr. C. E. Test, was not indicative of an elevated excretion of these materials.

After these preliminary observations the patient was given 25 mg. of testosterone propionate[1] intramuscularly on alternate days (Table 1). As a result of this therapy

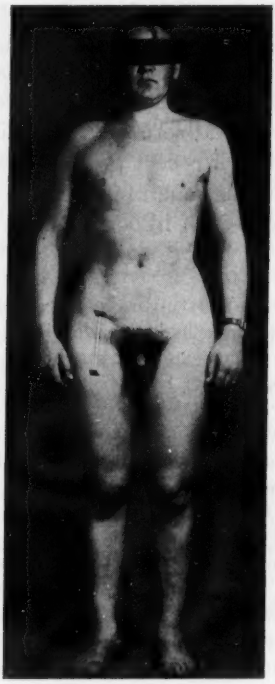

FIG. 1. L. K. (age 28 years).

his voice deepened and facial hair growth increased slightly (shaving frequency, three times a week). There was probably a minimal increase in the size of the prostate gland. Although no somatic complaints were elicited prior to treatment, he thought that his strength and vigor were improved by the androgen. Accompanying these changes the seminal fructose concentration rose promptly from 33 and 54 mg. per 100 ml. to 136 mg. per 100 ml. and remained between 162 and 264 mg. per 100 ml. during the remainder of the four months of testosterone propionate administration.

No treatment was given during the following nineteen months and during the first sixteen months of this period the patient noticed no change in his condition. The seminal fructose concentration was maintained at the treatment level for approximately a year,

[1] Testosterone propionate (Oreton) and methyl testosterone (Oreton-M) were generously supplied by Dr. Edward Henderson of the Schering Corporation.

TABLE 1

Date	Fructose conc. (mg./100 ml.)	Volume of semen (cc.)	Spermatozoa per ml.	Total spermatozoa	Treatment* and remarks
Mar. 10, '47	33	0.2	64,000,000	12,800,000	No treatment.
Mar. 22, '47	54	0.3	90,000,000	26,000,000	
Apr. 7, '47	136	0.6	370,000,000	222,000,000	Testosterone propionate, 25 mg.
Apr. 21, '47	173	2.0	420,000,000	840,000,000	I.m. every other day
May 5, '47	212	2.0	448,000,000	898,000,000	
June 30, '47	264	1.5	25,000,000	37,500,000	
July 14, '47	162	1.5	1,600,000	2,400,000	
July 28, '47	256	1.5	50,600,000	76,200,000	
Aug. 11, '47	240	1.5	40,000,000	60,000,000	No treatment.
Aug. 25, '47	193	1.5	3,800,000	5,700,000	
Oct. 6, '47	201	1.5	110,000,000	165,000,000	
Oct. 20, '47	227	1.5	160,000,000	140,000,000	
Nov. 10, '47	186	2.0	300,000,000	600,000,000	
Dec. 15, '47	235	1.5	209,000,000	314,000,000	
Feb. 27, '48	221	2.0	150,000,000	300,000,000	
Apr. 18, '48	170	2.0	190,000,000	380,000,000	
Aug. 11, '48	125	0.5	463,000,000	231,500,000	
Dec. 4, '48	60	0.5	530,000,000	265,000,000	Noticed recession of secondary sex
Jan. 8, '49	71	0.5	490,000,000	245,000,000	characteristics, and fatigue.
Feb. 19, '49	93	1.5	300,000,000	450,000,000	
Mar. 26, '49	136	2.5	no value	no value	Methyl testosterone, 10 mg./day
May 7, '49	161	2.0	185,000,000	370,000,000	Methyl testosterone, 10 mg./day
June 11, '49	178	2.5	152,000,000	380,000,000	Methyl testosterone, 10 mg./day
July 2, '49	254	no value	7,400,000	no value	Methyl testosterone, 25 mg./day
July 30, '49	244	no value	no value	no value	Methyl testosterone, 25 mg./day
Sept. 17, '49	204	2.0	1,100,000	2,200,000	Methyl testosterone, 25 mg./day
Oct. 29, '49	187	2.0	300,000,000	600,000,000	Testosterone propionate (sublingual), 5 mg./day
Jan. 14, '50	245	no value	no value	no value	Methyl testosterone, 25 mg./day
Feb. 25, '50	268	2.0	140,000,000	280,000,000	Methyl testosterone, 25 mg./day
Mar. 25, '50	181	1.5	320,000,000	480,000,000	No treatment; less physical vigor.
May 10, '50	182	2.0	27,000,000	54,000,000	
July 31, '50	254	1.5	120,000,000	180,000,000	
Aug. 30, '50	266	1.5	70,000,000	105,000,000	
Jan. 5, '51	145	1.0	230,000,000	230,000,000	
May 8, '51	170	1.5	130,000,000	195,000,000	

* All changes in treatment took place on the day following the date of the last ejaculate obtained during preceding regimen.

declining to 125 mg. per 100 ml. at this time and reaching 60 mg. per 100 ml. approximately coincident with L. K.'s unprovoked observation that he tired more easily and shaved less frequently. Two subsequent ejaculates had fructose concentrations in the same hypogonadal range. At this time testicular biopsies were performed.

With the reinstitution of androgen replacement therapy (10 mg. daily of methyltestosterone[1] given orally) he again felt more energetic, and the seminal fructose concentration rose to 136, 161, and 178 mg. per 100 ml.—approximately the same level attained previously during testosterone propionate therapy. An increase in the dosage of methyltestosterone to 25 mg. per day led to slightly higher values, but the significance of the difference is doubtful. This course of androgen lasted approximately one year, following which the patient was observed for sixteen months. Soon after the androgen was discontinued he complained of diminished stamina, but within several weeks this lessened

and disappeared entirely. The seminal fructose concentrations were unaltered, with the possible exception of a downward drift suggested by the last two values, observed eleven and fifteen months after treatment.

Sexual activity was never affected by the treatment or its withdrawal. He married approximately a year after the end of the second treatment period and his wife has borne one healthy infant.

Although the very low seminal volumes noted prior to androgen therapy support the diagnosis of hypogonadism, the volume of ejaculates did not provide the clear-cut reflection of androgen influence brought out by the shifts in fructose concentration. The seminal volumes tended to be lower toward the end of the 18-month interval without treatment, and increased again on the resumption of androgen, but the very low volumes obtained initially did not recur.

As previously indicated, the concentration of spermatozoa in the pretreatment ejaculates was normal (64 and 90 million per ml.); nevertheless, both the concentration and total content rose sharply during testosterone propionate therapy to a peak of 448 million per ml., only to drop while still receiving treatment to a distinctly lower level (one value of 1.6 million per ml. being obtained). The first 2 post-treatment ejaculates contained 40 and 3.8 million sperm per ml., but the counts in all of the remaining 10 specimens taken between treatment periods were normal, ranging from 110 to 530 million per ml. During treatment with 10 mg. of methyltestosterone per day, no change was noted, but the first 2 ejaculates obtained while the patient was receiving 25 mg. per day of methyltestosterone were again very low (1.1 and 7.4 million per ml.). After six weeks of therapy with 5 mg. per day of testosterone propionate sublingually, the methyltestosterone was resumed in the previous dosage, and the only ejaculate counted contained 140 million sperm per ml. With the exception of one rather low count of 27 million per ml., the sperm concentration remained well above 60 million per ml. (the frequently stated lower limit of normal) during the second post-treatment period. Throughout the study the spermatozoa in ejaculates exhibited normal motility (60 to 90 per cent) and the examinations of occasional stained smears failed to disclose an unusual percentage of abnormal appearing spermatozoa.

The testicular biopsy specimen (Fig. 2) was described by Dr. Eleanor Humphreys as follows:

> "*Gross:* The specimens are two nondescript small biopsies labelled left (larger) and right (very small). These were fixed at once in Bouin's solution.
>
> *Microscopic:* Distortion and displacement of cells makes it difficult to be sure of the luminal contents and interstitium in these testes biopsies. In the left testis most of the tubules are large, well formed, with thin basement membranes and good maturation sequences. The cells include many spermatids and at least a few spermatozoa. Two or three tubules seem to have slightly thickened basement membranes. Interstitial cells are few (but probably are normal in number and character). The right biopsy is smaller with more artefacts, but differs in no essential details.
>
> *Impression:* Testes biopsies with no significant abnormalities. (Mature sperm and interstitial cells are less readily demonstrated than in some of our biopsies. That chance can determine such sparseness is obvious from a study of large sections.)"

DISCUSSION

Taken alone, the physical signs of an androgen deficiency in L. K. might well be judged insufficient to be certain of the diagnosis of hypogonadism.

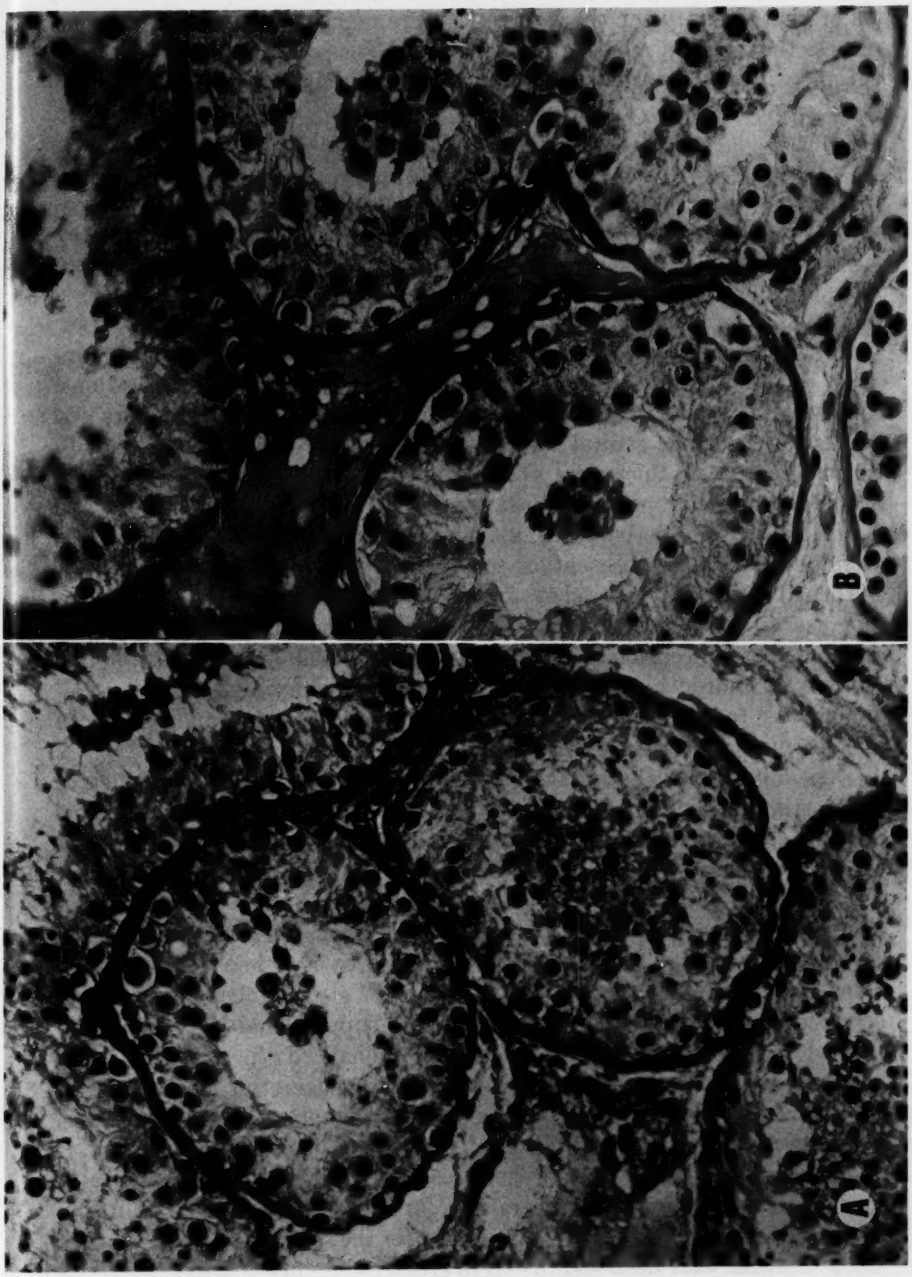

FIG. 2. Representative views from testicular biopsy specimens. a) ×390. b) ×475.

All of his secondary sex characteristics were manifest, though in most instances they did not appear fully developed. The possibility of an extreme variant within the normal range would undoubtedly come to mind under these circumstances. Accordingly, the finding of unusually low fructose concentrations in pretreatment ejaculates, followed by a highly significant rise during replacement androgen therapy, provided the necessary solid confirmation. With these clinical and laboratory indications of hypogonadism, the impression from the examination of the testicular biopsy specimen that fewer than the normal number of Leydig cells were seen, may be conditionally accepted as the histologic sign of the responsible testicular defect.

In spite of the hypogonadism, spermatogenesis did not seem to be materially impaired. The initial ejaculates showed normal sperm concentrations, and with such small fluid volumes many spermatozoa undoubtedly did not escape from the genital tract. At no period were the spermatozoa abnormal insofar as they were examined. The testicular biopsy specimen, secured after the influence of the first course of testosterone propionate had been lost, showed active spermatogenesis without any grossly abnormal tubule sections. This observation, together with the fact that the pregnancy of the patient's wife occurred over a year after the last hormone administration, serves as the ultimate proof of his reproductive capacity. It is realized that conception may not have been a possibility prior to the two courses of androgen replacement therapy. One can only state that, although the total number was deficient, the concentration and quality of the spermatozoa were satisfactory.

Depending upon the dosage employed, androgen showed evidence of either stimulating or inhibiting spermatogenesis in this patient. The initial spurt in spermatozoa output as a result of testosterone propionate was followed by a sharp decline to abnormally low levels, from which recovery did not occur until two months after treatment was stopped. Again, after spermatogenesis was well maintained on 10 mg. of methyltestosterone per day, an increase to 25 mg. daily was followed by a rather sharp suppression. If an optimum amount of androgen for spermatogenesis were not secreted by L. K.'s testes, it is reasonable to suppose that the administration of replacement quantities may have enhanced the activity of the seminiferous epithelium. As has been shown under other circumstances (10), excessive androgen might be expected to suppress spermatogenesis, presumably through an inhibition of pituitary gonadotropin secretion.

The enduring effect of androgen demonstrated by the persistence of normal seminal fructose concentrations for a year after the first course of treatment and for somewhat over a year after the second one, suggests that the testes may have been stimulated to greater secretory activity as a result

of androgen administration. These results had a parallel, however, in an earlier study in a eunuchoid in whom a shift from 25 to 10 mg. per day of methyltestosterone was followed by a four-month prolongation of a fructose concentration significantly greater than that previously characteristic of the 10 mg. dosage (5). This was ascribed to a continuation of the influence of the large amount of androgen when shifting the dosage downward, and might be explained by the need of less androgen for maintenance of a stated level of seminal vesicular activity than was required for its induction. Similarly, in this case, the small amount of endogenous androgen may have been sufficient to maintain normal fructose concentrations for a time, but the extension of this effect for over a year points toward an enhanced secretion of testicular androgen as a partial explanation.

No evidence for a gross pituitary defect could be adduced in this patient, but the studies of McCullagh and Beck (3) in similar patients suggested a deficiency in the secretion of pituitary interstitial-cell-stimulating hormone. The urinary excretion of follicle-stimulating hormone was normal in their patients and the excretion of interstitial-cell-stimulating material was too small to be measurable. The androgenic effect of chorionic gonadotropin which they reported supports the concept that the interstitial cells remained responsive to the proper gonadotropin. Nevertheless, the possibility remains, at least in our patient, that the primary defect may lie in a testicular abnormality in which the patient's Leydig cells are unable to respond fully to his own gonadotropin.

SUMMARY

A patient is described in whom a mild hypogonadism, which was confirmed by an unusually low seminal fructose concentration, was associated with intact spermatogenesis. Androgen substitution therapy resulted in a rise in seminal fructose concentration, the completion of masculine sex characteristics, and at least a temporary increase in the output of spermatozoa. The beneficial effects of androgen replacement lasted for one year after the first course of treatment and for at least sixteen months after the second course.

Acknowledgment

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REFRACTORINESS TO ANTITETANIC THERAPY IN A CASE OF SURGICAL HYPOPARATHYROIDISM

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THE advent of dihydrotachysterol entirely revolutionized the treatment of hypoparathyroidism. According to Albright and Reifenstein (1) this agent is administered in a dosage of 3 cc. daily until calcium appears in the urine, at which time the dosage is decreased to a maintenance level of approximately 1 cc. three to seven times weekly, depending upon the degree of hypercalciuria as determined by the Sulkowitch test. Additional measures, such as a high calcium and low phosphorus intake, and the administration of aluminum hydroxide, vitamin D, ammonium chloride and desiccated thyroid have frequently proved useful. Of these, vitamin D or vitamin D_2 (calciferol) have been used with particular frequency and are generally considered as effective as dihydrotachysterol in the management of chronic tetany. The following case of hypoparathyroidism is deemed worthy of reporting because of refractoriness to conventional antitetanic therapy, including large doses of dihydrotachysterol, and massive doses of vitamin D.

CASE REPORT

The patient was a 30-year-old housewife, who was admitted to Letterman Army Hospital in April 1950 with a six-month history of symptoms of irritability, intolerance to heat, an 8-pound weight loss despite a tremendous appetite, and occasional diarrhea.

Physical examination revealed a tachycardia of 100 per minute, fine tremor of the hands, bilateral exophthalmos, and a diffusely enlarged, soft thyroid gland. Laboratory findings included a basal metabolic rate of +35 per cent and the following values in serum: cholesterol 111 mg., protein-bound iodine 12.5 micrograms, calcium 11.6 mg., and phosphorus 4.6 mg. per 100 cc.

The patient was prepared for surgery by bed rest, sedation, and the administration of propylthiouracil and Lugol's solution. On June 6, 1950, a bilateral subtotal thyroid-

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ectomy was performed without technical difficulty. Thirty-six hours postoperatively severe carpopedal spasm developed, relieved by the administration of calcium intravenously. Chemical analysis of the blood revealed the calcium level to be 6.8 mg. and the phosphorus level, 5.4 mg. per 100 cc. The result of a Sulkowitch test on the urine was negative. Examination of the excised thyroid tissue revealed the inclusion of one parathyroid gland.

Initially, therapy consisted of a low phosphorus diet, with the administration of calcium lactate and Amphojel, plus 3 cc. of dihydrotachysterol and 10,000 units of vitamin D daily. After increasing the dose of dihydrotachysterol to 5 cc. daily, the serum

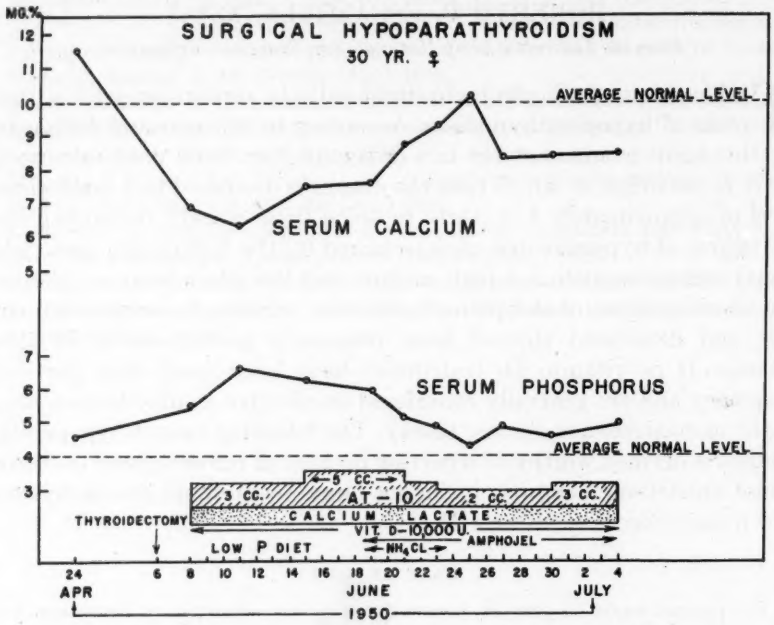

FIG. 1. Serum calcium and phosphorus levels, demonstrating initial favorable response to antitetanic therapy during first hospital admission.

calcium level rose and the patient was improved. The dosage was dropped to 3 cc. and then to 2 cc. daily, and improvement continued for four days with the concentration of serum calcium reaching 10.4 mg. and that of serum phosphorus decreasing to 4.2 mg. per 100 cc. The results of Sulkowitch tests on the urine had become positive, showing a trace to 1 plus of calcium. Subsequently, however, there was a fall of the serum calcium, necessitating resumption of a larger dosage of 3 cc. daily, and occasional injections of calcium salts to prevent tetany (Fig. 1). The patient was discharged from the hospital for outpatient follow-up on a daily regimen of 3 cc. of dihydrotachysterol, 10,000 units of vitamin D, and supplemental oral calcium lactate.

As an outpatient, she continued to manifest latent tetany, with increasing frequency of severe carpopedal spasms. After a dosage of 5 cc. of dihydrotachysterol daily failed to control her symptoms, she was readmitted to the hospital on August 17, 1950.

At the time of readmission, the concentration of serum calcium ranged from 5.8 to 7.5 mg. per 100 cc., and of serum phosphorus from 5.6 to 6.8 mg. per 100 cc. In an attempt to achieve control of symptoms, the dose of dihydrotachysterol was increased by 1 cc. every three days until a maximum of 17 cc. daily was reached. Adjuvant therapy at this time included vitamin D in progressively increasing increments up to a maximum of 100,000 units daily, a low phosphorus and low calcium diet, 36 grams of calcium lactate orally, and 4 grains of desiccated thyroid daily. Despite this therapy the patient continued to demonstrate latent tetany, and severe carpopedal spasms occurred once or twice weekly. Serum calcium levels averaged approximately 7 mg. per 100 cc. and serum phosphorus levels, 6 mg. per 100 cc. During this period, 3 to 4 loose stools per day occurred from two to three times weekly.

In October 1950, a controlled metabolic study was begun. A diet was given, estimated to contain approximately 0.65 Gm. of phosphorus and 0.17 Gm. of calcium daily, with the daily supplemental administration of 16.0 Gm. of calcium lactate orally, 4 grains of desiccated thyroid, and 200,000 units of vitamin D. Daily serum calcium and phosphorus levels were determined, and quantitative 24-hour urinary calcium and phosphorus excretions were estimated. Four-day stool collections were analyzed for calcium and phosphorus. After seven days, the serum calcium level was 7.5 mg. and the serum phosphorus, 4.9 mg. per 100 cc. The patient was then given 3 cc. of parathormone daily by deep intramuscular injection. A loss of muscle irritability and a sensation of well being resulted within thirty-six hours. There was an increase in the urinary phosphorus excretion, a decrease in the serum phosphorus level, and an increase in the serum calcium level. Results of the Sulkowitch tests became mildly positive. On this regimen, the concentration of serum calcium rose to 8.7 mg. per 100 cc. However, the parathormone injections caused considerable local pain, even when procaine was added; they were discontinued after twenty-three days. Within five days thereafter, muscle irritability returned and the serum calcium level had fallen to 7.1 mg., whereas that of serum phosphorus had risen to 5.7 mg. per 100 cc. On the same regimen, except for a reduction of vitamin D intake to 2,000 units daily, a dose of 10 cc. of dihydrotachysterol daily was prescribed on November 8, 1950. A mild increase in the concentration of serum calcium resulted, but there was no significant change in that of serum phosphorus (Fig. 2). The highest serum calcium level obtained was 8.0 mg. per 100 cc., and the serum phosphorus level ranged between 5.3 and 5.7 mg. per 100 cc. Muscle irritability, however, was less marked and severe carpopedal spasms were less frequent. There was no diarrhea with this trial of dihydrotachysterol. An attempt to reduce the dosage of dihydrotachysterol to 5 cc. daily resulted in marked muscle irritability, and markedly adverse serum calcium and phosphorus levels. Increasing the dosage to 10 cc. daily did not improve the serum calcium and phosphorus levels, but the patient felt somewhat better, although continuing to exhibit latent tetany.

At the end of the metabolic study (Fig. 3), dihydrotachysterol was stopped and was replaced by large doses of vitamin D for maintenance therapy. The patient was discharged to be followed in the Endocrine Clinic. Initially, 500,000 units of vitamin D were administered daily. This dosage was subsequently raised gradually to 1,000,000 and eventually to 1,500,000 units daily. A dairy-product-free diet, supplemental calcium lactate, aluminum hydroxide, and desiccated thyroid completed the regimen.

The patient became pregnant in January of 1951, but the chronic tetany continued with frequent numbness and twitching and attacks of carpopedal spasm. The serum calcium level rose slightly to 8.2 mg. per 100 cc. during the third month, with slight clinical improvement. The level of vitamin D dosage was kept at 600,000 units. However, the

patient miscarried at four months on April 7, 1951. Thereafter the frequency and severity of the tetany increased, so that intravenous calcium was necessary occasionally. The vitamin D dosage was then gradually increased, reaching 1,000,000 units on June 26, 1951. There was slight improvement as the dose was increased but on May 31, 1951 the serum calcium level was 7.1 mg. and the phosphorus, 4.3 mg. per 100 cc. However, she did fairly well, unless under stress. An ankle fracture during the summer of 1951 required four intravenous injections of calcium over a short period of time, to control severe tetanic symptoms. Meanwhile, she had again become pregnant, and again there resulted some amelioration of symptoms. On August 22, 1951 the serum calcium level was 8.2 mg. and the phosphorus level, 3.8 mg. per 100 cc. Her regimen was continued throughout the pregnancy, with the daily intake of calcium lactate increased to 4 tablespoonfuls three or four times daily. A bout of diarrhea in December 1951 caused an increase in tetany and on December 17, 1951 the serum calcium level was 7.3 mg. and the phosphorus level, 4.6 mg. per 100 cc. With increased activity over the Christmas holidays, symptoms were more marked for a time, and were finally helped by an injection of intravenous calcium. On February 15, 1952, x-ray films of the abdomen showed a normal-appearing fetus without evidence of calcification.

On the morning of March 5, 1952, the patient went into labor and delivered a normal female infant at 1:00 A.M. on March 6. With the start of labor, she began to take extra calcium lactate, ingesting 3 tablespoonfuls every thirty to forty-five minutes throughout the day. Only one intravenous injection of calcium was necessary, about half an hour

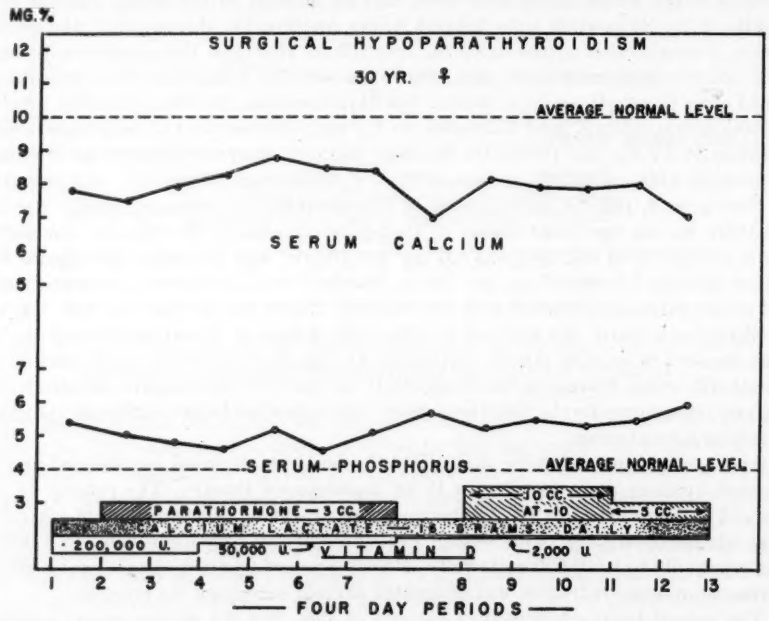

FIG. 2. Serum calcium and phosphorus levels, demonstrating response to parathyroid extract administration, and inadequate response to large doses of dihydrotachysterol.

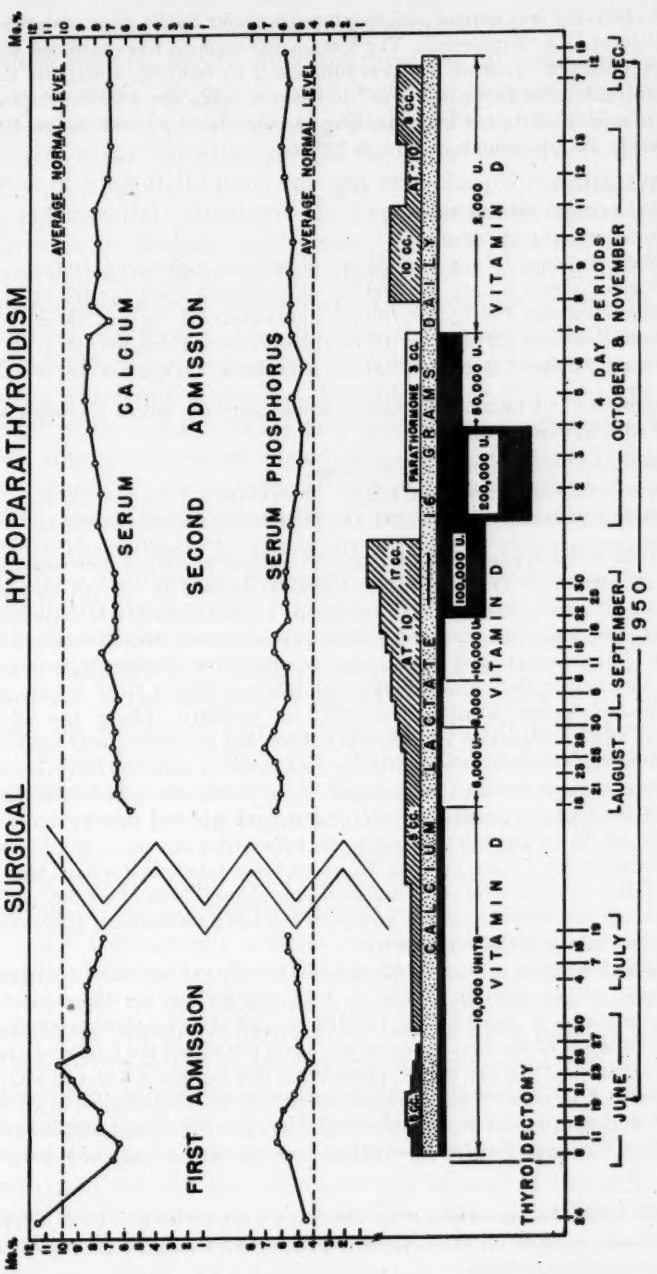

Fig. 3. Patient's course, including both hospital admissions, demonstrating initial favorable response to dihydrotachysterol during first hospital admission, subsequent loss of control and inadequate response of the serum calcium and phosphorus to massive doses of dihydrotachysterol (maximum 17 cc. daily), and partial response to parathyroid hormone and vitamin D in moderate dosage.

before the birth. Delivery was normal and spontaneous under saddle block anesthesia, supervised by Colonel E. A. Zimmerman. The therapeutic regimen was continued without interruption. Lactation occurred, but was suppressed by binding. About two days post partum, the patient began to feel "low" in calcium, with the tetanic symptoms becoming more prominent and more frequent. This was borne out by blood calcium studies and the necessity for intravenous calcium as follows:

March 5 Parturition.
March 7 Intravenous calcium twice.
March 12 Intravenous calcium once.
Serum calcium 7.7 mg. per 100 cc.; phosphorus 4.85 mg. per 100 cc.
March 14 Serum calcium 8.2 mg. per 100 cc.; phosphorus 5.0 mg. per 100 cc.
March 15 Serum calcium 7.8 mg. per 100 cc.; phosphorus 5.4 mg. per 100 cc.
March 16 Serum calcium 7.2 mg. per 100 cc.; phosphorus 5.5 mg. per 100 cc.
March 17 Serum calcium 7.0 mg. per 100 cc.; phosphorus 5.0 mg. per 100 cc.

Considerable numbness and tingling, frequent cramps, and very active Chvostek and Trousseau signs were apparent, and on

March 23 Intravenous calcium was given once.
March 24 Serum calcium 7.5 mg. per 100 cc.; phosphorus 4.5 mg. per 100 cc.
March 28 Serum calcium 6.9 mg. per 100 cc.; phosphorus 5.1 mg. per 100 cc.

On April 1, 1952 it was apparent that the patient was not as well as previously, and the daily dose of vitamin D was increased to 1,500,000 units. The regimen otherwise was the same, *i.e.*, calcium lactate 5 to 8 tablespoonfuls daily, Amphojel 3 tablespoonfuls three times daily, and desiccated thyroid 0.3 gram (5 grains) daily, in addition to a low phosphorus diet. Three weeks later the patient reported considerable improvement. She stated that the cloud in the morning Sulkowitch test was slightly more definite, and a blood serum specimen showed a calcium level of 9.2 mg. per 100 cc., phosphorus 4.2 mg. per 100 cc. and alkaline phosphatase 3.9 Shinowara, Jones and Reinhart units.[1] Examination at this time showed no trophic changes in hair, skin, teeth or nails and careful examination of the lens of the eyes revealed no opacities. Three weeks later (after six weeks on a dosage of 1,500,000 units of vitamin D daily), the patient reported that the improvement had continued. There was less tingling in the extremities and much less frequent spasm and she seemed generally less nervous. However, blood taken on this day (May 13, 1952) showed a serum calcium level of 6.9 mg. and a phosphorus level of 4.4 mg. per 100 cc. The Chvostek sign was still very active, and there was still a tendency to demonstrate a Trousseau sign on taking the blood pressure.

The female infant weighed 6 pounds, 12 ounces at birth and appeared completely normal. Her serum calcium level on March 11, 1952, was 9.1 mg. per 100 cc. and on March 12 serum phosphorus was 5.65 mg. per 100 cc. and alkaline phosphatase 13.95 S. J. & R. units. On April 22 the infant's serum was again tested and the following values were reported: calcium 11.1 mg. per 100 cc., phosphorus 10.8 mg. per 100 cc. and alkaline phosphatase 9.2 S. J. & R. units.[1] Another blood test on May 13, 1952 resulted in a serum calcium level of 10.1 mg. per 100 cc., phosphorus 7.1 mg. per 100 cc. and alkaline phosphatase 20 S. J. & R. units. Further observation is indicated for signs of hyperparathyroidism.

[1] On April 22, 1952, the laboratory determinations were performed by a different technician, which may account for these results, which were somewhat out of agreement with previous and subsequent tests.

DISCUSSION

Since the first use of dihydrotachysterol (2) numerous reports (3–21) of the successful use of this agent in the treatment of hypoparathyroidism have appeared in both the European and American literatures. Subsequently, many investigators (22–32) have demonstrated the favorable effects of vitamin D upon calcium and phosphorus metabolism in both the experimental animal and the hypoparathyroid human subject. Holtz (33), without documenting specific instances, referred to occasional patients who were found to be hyposensitive to dihydrotachysterol; even with massive doses it was sometimes impossible or difficult to raise the serum calcium level and to eliminate the clinical symptoms. Martini and Heymer (34) reported a patient with chronic postoperative hypoparathyroidism who required doses of A.T. 10 varying from 10 to 15 cc. daily for several weeks before clinical and biochemical control was obtained, but whose subsequent course was not remarkable. Cutting, Wells, and Carnes (35) reported 1 case of surgical hypoparathyroidism that was refractory to 5 cc. of dihydrotachysterol daily for twenty-one days, with a few days of 10 to 15 cc. doses. Their patient fortunately experienced what presumably was a spontaneous remission, and was well one year postoperatively without any medication.

The reason for the refractoriness of our patient to conventional antitetanic therapy is not clearly evident from a review of her history or from the results of the metabolic study (Fig. 3). Review of the data obtained during the periods of parathormone and dihydrotachysterol administration (Tables 1 and 2) suggests that the calcium-absorption effect of dihydrotachysterol was possibly greater than its phosphate-diuresis effect. However, there are discrepancies; *e.g.*, the fecal calcium excretion during periods 7 and 10, and the fecal phosphorus excretion during period 1 and possibly periods 9 and 12. Further, the magnitude of apparent calcium absorption is not consistent with the serum calcium levels of the patient, nor can the excessively positive calcium balance be related to bone salt deposition. The balance data thus do not support quantitatively the known facts of calcium and phosphorus metabolism. Except for demonstrating grossly that dihydrotachysterol was not an inert product, the figures do not contribute to the understanding of this patient's refractoriness. It is believed that her response to parathyroid extract in only moderate dosage was characteristic, and that larger dosage would have produced a more decisive change in the serum calcium and phosphorus levels. However, parathyroid extract was not continued because of the well known tendency of parathormone to cause the formation of antihormones, resulting in the loss of clinical effect after a period of time. Despite some lessening of the severity of the tetanic symptoms, however, adequate clinical or

TABLE 1. CALCIUM BALANCE DATA

Vitamin D was given as the sole agent during period 1. During the remaining periods vitamin D was paired with either parathormone or AT-10 in daily dosage as indicated. The data are expressed as 4-day totals, in grams.

Period	Treatment	Intake*	Excretion			Balance
			Fecal	Urinary	Total	
1	Vit.D, 200,000	9.480	2.720	Negl.	2.720	+6.760
2	Parathormone, 3 cc. Vit.D, 200,000	9.480	3.480	.100	3.580	+5.900
3	Parathormone, 3 cc. Vit.D, 200,000	8.990	3.040	.210	3.250	+5.740
4	Parathormone, 3 cc. Vit.D, 50,000	8.990	4.600	.410	5.010	+3.980
5	Parathormone, 3 cc. Vit.D, 50,000	8.990	4.600	.510	5.110	+3.880
6	Parathormone, 3 cc. Vit.D, 50,000	8.990	4.440	.740	5.180	+3.810
7†	Parathormone, 3 cc. Vit.D, 50,000	6.740	.720	.360	1.080	+5.660
		(1-week interval)				
8	AT-10, 10 cc. Vit.D, 2,000	8.860	1.030	.260†	1.290	+7.570
9	AT-10, 10 cc. Vit.D, 2,000	8.860	2.320	.300†	2.620	+6.240
10	AT-10, 10 cc. Vit.D, 2,000	8.860	.680	.320	1.000	+7.860
11	AT-10, 5 cc. Vit.D, 2,000	8.860	1.170	.280	1.450	+7.410
12	AT-10, 5 cc. Vit.D, 2,000	8.860	1.870	.290	2.160	+6.700
13	AT-10, 8 cc. Vit.D, 2,000	8.860	1.300	.190	1.490	+7.370

* Estimated dietary calcium plus 64 grams of calcium lactate per 4-day period (16 grams daily).

† Three days only.

Fecal calcium was determined by the method of Tisdall and Kramer, *J. Biol. Chem.* **48**: 1, 1921. Urinary calcium was determined by a modification of the method of Clarke and Collip, *J. Biol. Chem.* **63**: 461, 1925.

biochemical control was never achieved in this patient with dihydrotachysterol in a dosage up to 17 cc. daily, or with the massive doses of vitamin D.

TABLE 2. PHOSPHORUS BALANCE DATA

Vitamin D was given as the sole agent during period 1. During the remaining periods vitamin D was paired with either parathormone or AT-10 in daily dosage as indicated. The data are expressed as 4-day totals, in grams.

Period	Treatment	Intake*	Excretion			Balance
			Fecal	Urinary	Total	
1	Vit.D, 200,000	2.980	2.300	1.270	3.570	−.590
2	Parathormone, 3 cc. + Vit.D, 200,000	2.980	.310	2.080	2.390	+.590
3	Parathormone, 3 cc. + Vit.D, 200,000	2.580	.410	1.810	2.220	+.360
4	Parathormone, 3 cc. + Vit.D, 50,000	2.580	.660	1.710	2.370	+.210
5	Parathormone, 3 cc. + Vit.D, 50,000	2.580	.660	3.010	3.670	−1.090
6	Parathormone, 3 cc. + Vit.D, 50,000	2.580	.920	2.280	3.200	−.620
7†	Parathormone, 3 cc. + Vit.D, 50,000	1.940	.350	1.210	1.560	+.380
		(1-week interval)				
8	AT-10, 10 cc. + Vit.D, 2,000	2.960	.830	1.340†	2.170	+.790
9	AT-10, 10 cc. + Vit.D, 2,000	2.960	1.650	1.510	3.160	−.200
10	AT-10, 10 cc. + Vit.D, 2,000	2.960	.470	2.490	2.960	0.000
11	AT-10, 5 cc. + Vit.D, 2,000	2.960	.830	2.200	3.030	−.070
12	AT-10, 5 cc. + Vit.D, 2,000	2.960	1.470	1.730	3.200	−.240
13	AT-10, 8 cc. + Vit.D, 2,000	2.960	.680	2.210	2.890	+.070

* Estimated dietary phosphorus per 4-day period.

† Three days only.

Fecal and urinary phosphorus were determined by modifications of the method of Fiske and SubbaRow, *J. Biol. Chem.* **66**: 375, 1925.

Her failure to respond to activated sterol therapy may be considered under the following possibilities:

1) Failure of the patient to ingest the agents.
2) Failure of absorption.
3) Inactivation of the agents by some antigen-antibody mechanism.

4) Excessive demand.
5) Physiologic antagonism.
6) The pre-existence of secondary renal hyperparathyroidism.

Because of the initial favorable response to antitetanic therapy, our first conclusion was that the patient had ceased to take her medication. The possibility of her failure to ingest dihydrotachysterol was immediately eliminated by its supervised administration by hospital attendants.

The possibility of her refractoriness because of inadequate absorption from the gastro-intestinal tract has not been completely excluded. Holtz (33) stated that the cause of inadequate response to dihydrotachysterol in some patients was poor absorption from the gastro-intestinal tract, citing the experimental evidence of depancreatized dogs that failed to react to the oral administration of dihydrotachysterol except when desiccated or fresh pancreas was given simultaneously. Albright, Butler and Bloomberg (36) in their studies of rickets resistant to vitamin D therapy, showed that the patients did no better when the vitamin D (calciferol) was administered intravenously, and concluded that faulty absorption was not a factor. They administered vitamin D in doses up to 1,500,000 units daily. Jordan and Kelsall (37) attributed the improvement in control in their case of idiopathic hypoparathyroidism to an increased calcium absorption incident to an increased protein intake, together with a moderately large dose of calciferol daily.

The occurrence of mild diarrhea for two to three days out of each week during the administration of large doses of dihydrotachysterol suggested inadequate absorption in our patient. However, the absence of diarrhea during the metabolic study and the prolonged observation without gastro-intestinal disorder while the patient was receiving massive doses of vitamin D would tend to discount lack of absorption as a possibility. Further, during the metabolic study, observation and chemical examination of the stool for gross oil and abnormal fat content showed neither to be present. It seems unlikely, therefore, that inadequate absorption played a significant role.

The possibility of an allergic response to dihydrotachysterol was examined. The initial favorable response to the medication following surgery and lasting for approximately two weeks would provide the opportunity for sensitization. The occurrence of diarrhea subsequently when the dosage of dihydrotachysterol was increased might represent a gastro-intestinal allergic manifestation. There were, however, no associated colic, nausea, vomiting, melena or purpuric skin manifestations. A positive reaction to the skin test for dihydrotachysterol, as demonstrated by a central bleb and marked peripheral edema and erythema at forty-eight hours, lends interest to this hypothesis. Repetition of the skin test with the same ma-

terial upon 20 normal subjects gave negative results in all. So far as the authors know, there is no precedent for the indictment of dihydrotachysterol or other related sterols as an antigenic agent. It is of interest to recall that parathyroid hormone tends to become ineffective in the management of hypoparathyroidism because of its antigenicity. This substance, however, is a protein and bears no physio-chemical resemblance to the activated sterols.

Holtz (33), Schwartz *et al.* (38), Curtis (39), McLean (25), and Lyle (40) have pointed out the increased demands for, and the necessity for increasing the dosage of, dihydrotachysterol during pregnancy, lactation, and menstruation. Actual antagonism between estrogens and the activated sterols is implied by Holtz (33). More recently the antagonistic effect of estradiol to implants of vitamin D_2 has been reported (41). However, in our patient, in spite of mild cyclic variations in symptoms with menstrual periods, there was improvement during the two pregnancies and accentuation of symptoms after their terminations. Thus the basic refractoriness apparently cannot be explained by excessive demands or by physiologic antagonisms.

Lastly, the possibility of a pre-thyroidectomy secondary renal hyperparathyroidism is clearly excluded. The preoperative findings of a serum phosphorus level of 4.6 mg. per 100 cc. and a calcium level of 11.6 mg. per 100 cc. suggested the remote possibility of primary renal disease with insufficiency, acidosis, and a compensatory hypertrophy of the parathyroids in response to an initial low level of serum calcium. The removal of such parathyroids during surgery might result in a severe and possibly intractable tetany. However, adequate studies early in the patient's course showed no abnormality of renal function and microscopic examination of the excised parathyroid tissue showed nothing remarkable. It seems more likely that the preoperative serum calcium and phosphorus values reflected osteoporosis and demineralization. In this connection, roentgenograms showed no changes of the skeleton preoperatively, and roentgen studies near the end of the second pregnancy showed an apparently normal texture of bone.

Of some interest was the effect of stress in causing an exacerbation of tetanic symptoms. Any stressful episode, from ankle fracture to becoming overtired during Christmas activities, resulted in the need for intravenous calcium to control severe symptoms.

The improvement during the pregnancies was definite, though slight, being more marked with the full-term pregnancy. This improvement may have been due principally to a new source of parathormone—the parathyroid glands of the developing fetus. Moreover, the exacerbations of tetanic symptoms after the miscarriage and after the full-term pregnancy suggest

that when the fetal source of parathormone was removed, it was definitely missed. The fall in serum calcium concentration after delivery of the child (Fig. 4) also indicates this possibility. There is analogy here to the hypothyroid mother who is improved during pregnancy by the thyroxine from the thyroid of the fetus, or the diabetic mother whose diabetes is

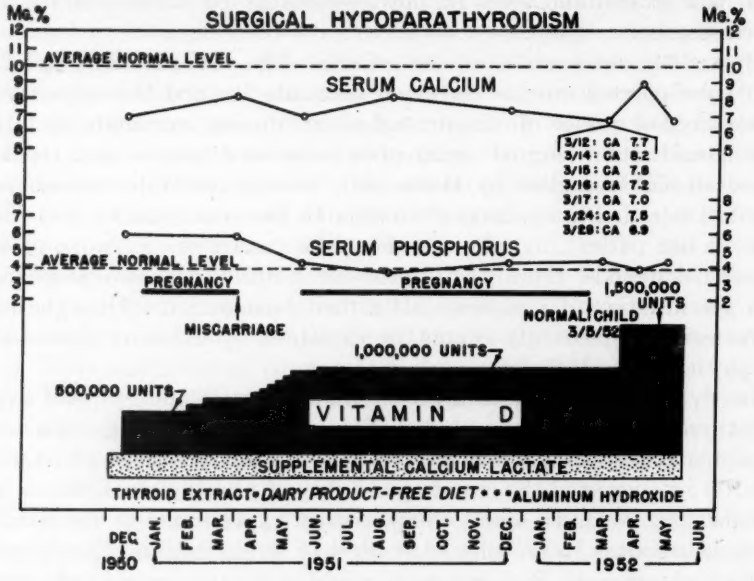

FIG. 4. Patient's course, demonstrating inadequate response of serum calcium and phosphorus to massive doses of Vitamin D (1,500,000 units daily). Although not clearly reflected in the graph, the patient was improved during her two pregnancies, and experienced a severe exacerbation of symptoms following miscarriage and delivery.

less severe due to the new source of insulin; in these conditions, the newborn child may show overactivity (*i.e.*, hyperthyroidism or hyperinsulinism) of the thyroid or of the pancreas that has been functioning for both mother and child in an effort to make up for the maternal deficiency. The obvious analogy in the present case is that the child must be observed for signs of hyperparathyroidism. To date, however, $2\frac{1}{2}$ months after birth, no such signs are apparent.

The use of vitamin D in massive doses is rare in this condition but has been reported with some frequency in vitamin D-resistant rickets. Pederson and McCarroll (42) used doses of vitamin D up to 600,000 units daily. Bakwin, Bodansky and Schorr (43) reported the use of doses up to 1,000,000 units daily and, as already mentioned, Albright, Butler and Bloomberg (36) gave up to 1,500,000 units daily. They all reported that the massive

doses caused healing of the rickets in the resistant cases and that later a smaller dose could be used for maintenance. However, even the "smaller" dose was relatively massive (100,000–300,000 units daily in children).

Since we still have not achieved good control in this patient, it is difficult to say if this observation will hold true in this instance of refractory hypoparathyroidism.

The trophic disorders seen occasionally in prolonged hypoparathyroidism include changes in the hair, skin, nails, and teeth, and the development of cataracts. So far, none of these has appeared in the subject of this report.

SUMMARY

The case is presented of a 30-year-old housewife, first hospitalized because of hyperthyroidism. Examination and study showed typical exophthalmic goiter, and following preparation with propylthiouracil and Lugol's solution a subtotal thyroidectomy was performed. Thirty-six hours postoperatively, frank tetany was manifest. One parathyroid gland was found in the excised tissue.

Treatment with diet, vitamin D, calcium, Amphojel and dihydrotachysterol was effective for two weeks, but control was subsequently lost and latent tetany with occasional episodes of severe carpopedal spasm appeared despite increased doses of dihydrotachysterol up to 17 cc. daily. Metabolic studies were carried out during a four-month period of observation. Parathormone elicited the characteristic response but dihydrotachysterol seemed ineffective in lowering the serum phosphorus and elevating the serum calcium significantly above control levels. The patient has been maintained on a dairy-product-free diet, oral calcium, Amphojel, and 1,500,000 units of vitamin D daily but continues to manifest latent tetany with occasional carpopedal spasms associated with a level of serum calcium averaging 8 mg. per 100 cc. and phosphorus, 5 mg. per 100 cc.

Two pregnancies have occurred during the period of observation. The first was terminated by miscarriage at four months, but the second went to term. In both instances, the patient was somewhat improved during the pregnancies, especially during the one that went to term, but there was exacerbation of the tetanic symptoms after delivery. The living infant seemed normal at birth, and after 2½ months has exhibited no signs of hyperparathyroidism. The mother has shown no trophic changes. Several possible causes for the refractoriness are considered but no adequate explanation can be offered.

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THE INFLUENCE OF SOME HORMONAL SUBSTANCES ON THE NITROGEN BALANCE AND CLINICAL STATE OF ELDERLY PATIENTS

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OUR early studies (1, 2, 3) on protein metabolism and nitrogen balance in elderly individuals have indicated that they are in less stable equilibrium than are young adults. Elderly subjects require a relatively larger amount of protein to maintain nitrogen balance and this balance is more readily disturbed by physiologic stress. It has also been found that when the balance is disturbed, a longer period of time is required to reestablish equilibrium.

We have recently indicated (4) that it is not always possible to obtain a satisfactory protein intake in elderly individuals. The use of protein-containing foods such as meat, eggs and milk to secure a higher protein intake, may be difficult. Patients may not tolerate the higher protein diet for the required length of time. We found, in fact, that in some instances attempts to increase the protein intake markedly, actually resulted in a decrease in the amount of total protein consumed. An alternative to increasing the protein consumption was to find a means of increasing the retention of the protein ingested. We showed that the administration of androgens will, under certain circumstances, increase the nitrogen retention of elderly subjects with a protein intake of 0.5 to 1.5 grams per kilogram of body weight per day. Therefore, it seemed advisable to study the influence of other hormones or hormone substances on the nitrogen balance in elderly individuals and at the same time note the influence of these substances on the general health.

It was thought that increasing the nitrogen retention by the administration of the proper hormones or combination of hormones, might prove to be more satisfactory than increasing the amount of protein ingested. Such studies might also indicate the extent to which the altered protein requirements in the elderly individual are due to, or influenced by, the presumed decrease in hormone secretions in such subjects.

A number of hormones and hormone substances have been administered

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by others to elderly subjects, either in general replacement therapy or in the treatment of recognized specific conditions. The study of the effect of these substances on the nitrogen balance and protein metabolism would indicate to what extent the protein metabolism may be altered during therapy with any particular hormone.

MATERIAL AND METHODS

The subjects of this study received androgens, estrogens, thyroid substance, insulin, cortisone and ACTH; also terramycin and Townsen-C (aluminum silicate) orally, and sterile saline hypodermically. Terramycin and Townsen-C have been shown in young animals to increase protein utilization and were used primarily to evaluate the nature of the disturbed nitrogen equilibrium seen by us in older people, and also to establish controls for our studies. The injections of sterile saline were used because we also wanted to rule out any psychic influence of the administration of medication and its influence on the nitrogen balance and on the welfare of the individuals.

The patients used in this study were inmates of the St. Louis City Infirmary Hospital and most of them had been used in some of our earlier studies (3, 4), to which we refer the reader for details of initial clinical evaluation of the subjects as well as the technique and methods used in conduction of the nitrogen balance studies. The patients were cooperative and not acutely ill. Unless otherwise indicated, they were on a well balanced diet furnishing 1 gram of protein and about 35 calories per kilogram of body weight per day. The intake of protein substance was closely adjusted to the desired level through the entire period of study, using the technique previously reported.

In addition to the determination of nitrogen balance, careful clinical observations were made on these patients. They were examined initially and daily throughout the period of study. In addition to the physical studies, observations were made on basal metabolic rate, glucose tolerance, electrocardiogram, red and white blood cell count, blood pressure and, during the time the patients were receiving ACTH, the eosinophil count. These tests were made periodically, but especially before and toward the end of the period during which the patient was receiving a specific medication.

RESULTS

Androgens and estrogens

Since we have given a preliminary report on the influence of androgen and estrogen on the nitrogen balance and intend to report further on these substances at a later time, we shall mention only briefly the findings with

these substances. It has been found that testosterone, 25 mg. three times weekly intramuscularly, in association with any diet level of protein varying from 0.5 Gm. to 1.5 Gm. per kilogram of body weight per day, will cause a decided increase in nitrogen retention. This is true in both males and females. The estrogens, on the other hand, cause a moderate nitrogen retention only when the individuals are on a low protein intake of 0.5 Gm. per kilogram of body weight per day. When the protein intake is increased to 1 Gm. or more, little or no effect is noted from the administration of estrogen.

This paper will otherwise confine itself to a report on the effect of thyroid, cortisone, insulin, ACTH, Terramycin, Townsen-C and placebos on the nitrogen balance and on the observed clinical state of the individual while taking the substances.

Thyroid substance

Table 1 indicates the effect of the administration of small doses of thyroid substance on the nitrogen balance. The nitrogen balances are all expressed

TABLE 1. EFFECT OF THYROID ADMINISTRATION ON NITROGEN BALANCE

Subject	Dosage per day		Average nitrogen balance in mg./Kg./day		
			Administration of thyroid		
	gr.	mg.	Before	During	After
M.C.	¼	16	17	16	16
O.S.	¼	16	34	42	
O.S.*	¼	16		42	33
D.E.	½	32	21	22	19
A.D.	½	32	13	20	
A.D.*	½	32		33	29
M.A.	¾	48	33	34	34
T.A.	¾	48	25	24	
K.I.*	1	65	28	29	24
K.O.	1	65	15	16	16

* Protein intake was 1.0 gram per kilogram of body weight per day, except when marked (*), indicating an intake of 1.5 grams per kilogram.

as milligrams of nitrogen retained per kilogram of body weight per day. The averages are usually for five 5-day periods. When the balance is given both before and after the thyroid administration for the same subject on the same diet, the average nitrogen balance during the administration of the hormone is taken over ten 5-day periods.

Figure 1 shows in detail the nitrogen balance for 1 subject (M.C.)

prior to, during, and after the administration of thyroid substance. The nitrogen balance is plotted for each 5-day period. The dotted lines indicate the over-all averages, before, during, and after the hormone administration. The data are typical of those for the other subjects, each of whom showed about the same variation from period to period in the nitrogen

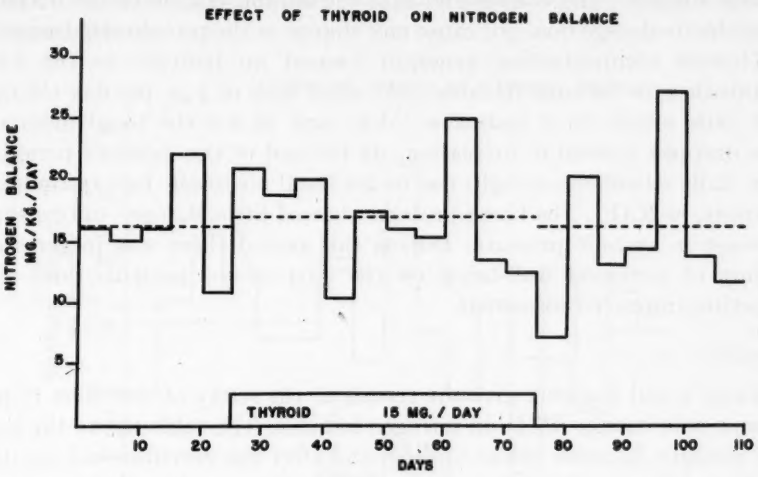

FIGURE 1

balances. In discussing the results of this portion of the study, it may be noted in Table 1 that of the 7 subjects on the protein intake of 1.0 Gm., 5 (M.C., D.E., M.A., T.A., and K.O., who received respectively $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, $\frac{3}{4}$, and 1 gr. of thyroid per day) showed no appreciable change in nitrogen balance with the administration of thyroid. The other 2 subjects (O.S., whose basal metabolism was low, and A.D.) initially showed some additional retention of nitrogen upon administration of thyroid. The increase was approximately the same in the 2 patients, one of whom received $\frac{1}{4}$ gr. of thyroid and the other $\frac{1}{2}$ gr. These 2 subjects were also studied on an intake of 1.5 Gm. of protein per Kg. One (A.D.) showed a marked increase in nitrogen retention due to the change in diet, in an earlier study. On the higher protein intake level this subject showed no additional change due to the thyroid medication. The other subject (O.S.) showed no change (during the earlier study) in nitrogen balance due to the higher protein intake level, but at both low and high protein intake levels showed an increase in nitrogen retention due to the thyroid administration.

The final subject of the group (K.I.) was studied only on a protein intake of 1.5 Gm. per kilogram. This subject had previously showed a negative nitrogen balance on an intake of 1.0 Gm. of protein, and any

change in nitrogen retention shown in the table may be due more to the adjustment of the subject to the diet than to any effect of thyroid administration. From the results obtained over the period of time studied, we may conclude that administration of up to 1 grain of thyroid per day will not usually result in any significant increase in the nitrogen retention in elderly subjects; and conversely, that the administration of the hormone in moderate dosage does not cause any change in the protein requirements.

Thyroid administration generally caused an increase in the basal metabolic rate. In most instances, the small dose of $\frac{1}{4}$ gr. per day (16 mg.) had little effect. In 2 instances (M.C. and M.A.) the basal metabolic rate dropped instead of increasing. At the end of the 2-month period on 1 gr. daily, there was a slight rise in the basal metabolic rate (as much as 6 points, in K.O.). The blood pressure showed little change—only a slight increase in systolic pressure. During this period there was in general a feeling of increased well-being on the part of the patients, and their appetites improved somewhat.

Insulin

Table 2 and Figure 2 give the results of the study of the effect of protamine-zinc insulin (PZI) on nitrogen balance. The table shows the average nitrogen balances before, during, and after the subcutaneous administration of 5 units of insulin per day, or 10 units per day, the doses being given shortly before the noon meal. All of the subjects had an intake of 1.0 Gm. of protein per kilogram. The nitrogen balances are again expressed as milligrams of nitrogen per kilogram of body weight per day.

TABLE 2. EFFECT OF INSULIN ON NITROGEN BALANCE

Subject	Dosage* (units) PZI	Average nitrogen balance in mg./Kg./day		
		Administration of insulin		
		Before	During	After
A.D.	5	11	11	12
D.E.	5	18	34	23
M.A.	5 & 10	20	25	21
K.I.	5 & 10	0	2	4
K.O.	5 & 10	18	15	13
M.C.	5 & 10	15	19	13
B.U.	5	24	23	14

* 5 = 5 units per day for entire twenty days.
5 & 10 = 5 units per day for the first ten days, 10 units per day for the second ten days.

The averages are for six 5-day periods before the administration of the hormone, four 5-day periods during the insulin therapy, and six 5-day periods after treatment. Figure 2 shows the balances for the separate periods for 1 subject (M.C.). The average balances are shown by the dotted lines. Again, these data are typical of those for the other subjects, each of whom showed approximately the same period-to-period variation in nitrogen balance.

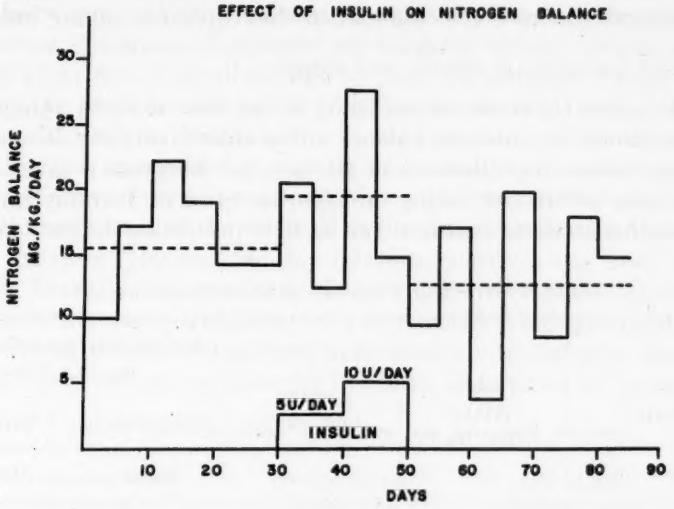

FIGURE 2

From the table, it will be noted that 2 of the subjects (A.D. and K.I.) showed but little change in the nitrogen balance with insulin administration. Three subjects (D.E., M.A., M.C.) showed an increase in nitrogen retention during the period of hormone administration. One subject (B.U.) showed no change when the administration of the insulin was begun, but did show a decrease in nitrogen retention when the insulin was discontinued.

For those subjects who received first 5 U. of insulin per day, and later 10 U. per day, the experimental periods were not long enough to show any consistent difference in nitrogen balance during the two dosages. Consequently, the average nitrogen balance for the whole period of hormone administration is listed in the table. The results indicate a slight but definite increase in nitrogen balance with insulin.

As a general rule, there was no change in the basal metabolic rate in the subjects receiving 5 and 10 U. of insulin per day. The glucose tolerance

curves before the administration of insulin showed a delayed return to normal levels in most of the patients, and in some the curve was flat. All of the curves became flat during the period of insulin administration. This reaction was particularly notable in those who received 10 U. of insulin per day. There was some increase in the appetites of these patients, particularly in those receiving 10 U. In Patient K.I. a response was noted that is commonly associated with hyperinsulinism, *i.e.*, sweating and nervousness before meals. The subjects complained less of being tired and were generally more active than when they were not taking insulin.

Terramycin, aluminum silicate, and placebo

Table 3 gives the results of our study on the effect of orally administered Terramycin on the nitrogen balance in the elderly subjects. The average nitrogen balance in milligrams of nitrogen per kilogram is given for 20-day periods, before and during the administration of Terramycin. It will be noted that tests were made at two levels of protein intake and two levels

TABLE 3. EFFECT OF TERRAMYCIN ON NITROGEN BALANCE

Subject	Protein intake (Gm./Kg.)	Dosage (mg./day)	Average nitrogen balance in mg./Kg./day	
			Administration of terramycin	
			Before	During
A.D.	1.5	250	28	22
M.A.	1.5	250	36	34
O.S.	1.5	50	34	30
K.I.	1.5	50	20	22
D.E.	1.0	250	20	17
K.O.	1.0	250	14	18
M.V.	1.0	50	17	17
B.U.	1.0	50	30	32

of Terramycin dosage. There were no significant changes in nitrogen balance during the Terramycin administration. In the 2 patients on a protein intake of 1.5 Gm. per kilogram of body weight per day, 250 mg. of Terramycin per day seemed to have the effect of slightly decreasing the nitrogen retention. There was also a slight decrease in the nitrogen retention in 1 of the 2 subjects ingesting 1.5 Gm. of protein and 50 mg. of Terramycin per day. In the subjects on the protein intake of 1.0 Gm., 250 mg. of Terramycin caused a decrease in the nitrogen retention in one subject and an increase in the other. A dose of 50 mg. of Terramycin per day

caused no change in the nitrogen balance on the lower protein intake. A similar response was noted to the injection of sterile saline and to the ingestion of Townsen-C (aluminum silicate).

Clinically, the patients showed no changes during administration of Terramycin or of Townsen-C.

Cortisone, and ACTH

Figures 3 to 8 indicate the effects of cortisone and ACTH on the nitrogen balance in our elderly subjects. The nitrogen balances are plotted as the average milligrams of nitrogen per kilogram of body weight per day for each 5-day period. In all the figures there is a period of 110 days (indicated by the break in the curves) which is not plotted. During this time the foregoing tests with insulin and Terramycin were performed. During all of the time represented in the figures, the subjects were ingesting 1.0 Gm. of protein per kilogram of body weight per day. During the first test with cortisone, 5 of the subjects, as illustrated by Figures 5 and 6, received the drug (100 mg. per day intramuscularly in one dose in midmorning) for one 5-day period only. Four subjects received the same dosage daily for twenty days. In all instances a marked fall in nitrogen balance was noted. The maximum decrease was approximately the same in those subjects who received the cortisone for five days, as in those who received it

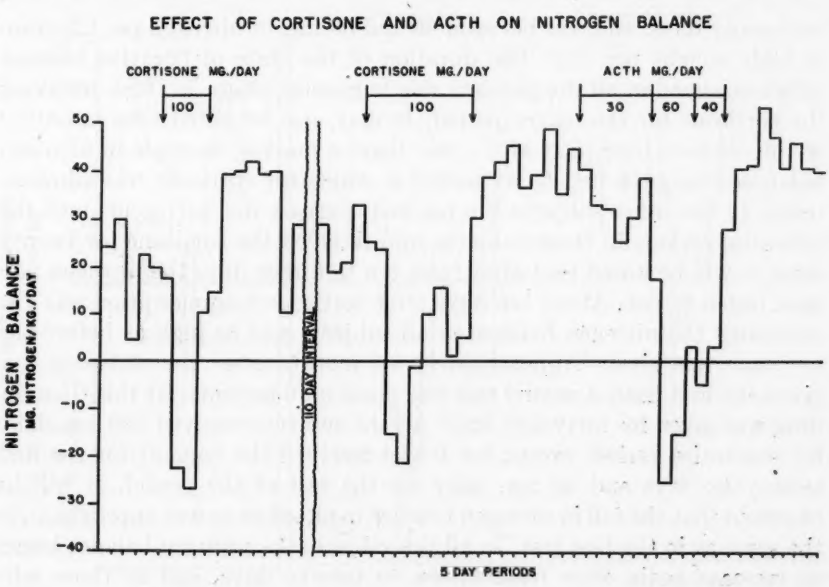

FIG. 3. (M.A.)

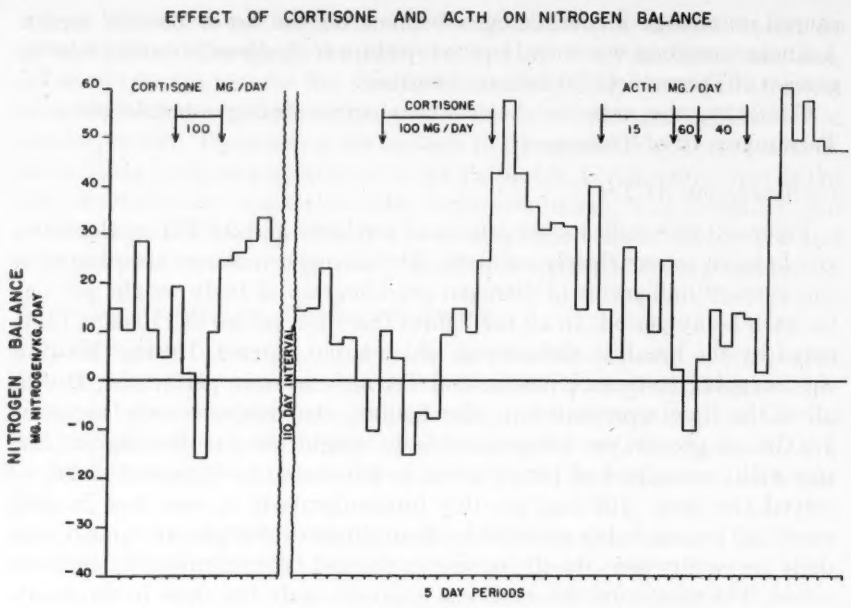

FIG. 4. (D.E.)

for twenty days, and was between 40 and 50 mg. of nitrogen per kilogram of body weight per day. The duration of the state of negative balance which occurred in all the patients was in general longer in those receiving the cortisone for the longer period. It may also be noted that in only 2 of the subjects (O.S. and M.C.) was there a marked decrease in nitrogen balance during the first 5-day period in which the cortisone was administered. In the other subjects the marked decrease did not occur until the following period. In those subjects who received the cortisone for twenty days, it will be noted that after from ten to fifteen days the nitrogen balance began to rise. About ten days after cortisone administration was discontinued the nitrogen balance in all subjects was as high as before the hormone was given. Approximately six months after the cortisone was given the first time, a second test was made on 6 patients. At this time the drug was given for forty-five days. All the subjects received 100 mg. daily for the entire period, except for 1 who received the amount for the first twenty-six days and 50 mg. daily for the rest of the period. It will be observed that the fall in nitrogen balance in all subjects was approximately the same as in the first test. In all the subjects the nitrogen balance began to increase again after from fifteen to twenty days, and in those who received 100 mg. of cortisone daily throughout the entire period a second-

ary fall in the nitrogen balance was observed during the seventh or eighth 5-day period. In all the subjects the nitrogen balance had reached its original level by the second 5-day period after the hormone administration was discontinued. In 4 of the subjects (M.A., D.E., O.S., K.O.), for a considerable period of time, the nitrogen balance remained elevated above the average value obtained before the cortisone administration. These 4 subjects experienced a rise in blood NPN of 10 to 15 mg. per 100 cc during the cortisone administration, whereas the other 2 subjects (M.C. and K.I.) had no rise in NPN. During this period the subjects were presumably regaining some of the lost protein.

The clinical effect of cortisone was studied on the two occasions of administration. The first period of administration was relatively brief and the tendencies were the same as those seen during the second period. The first clinical symptom noted was the development of a sense of well-being, occurring on the first or second day and lasting throughout the period. Some complaints were made of blurring of vision in the second week, particularly in those individuals who received 100 mg. per day. During the second week a sense of weakness associated with swelling of the legs

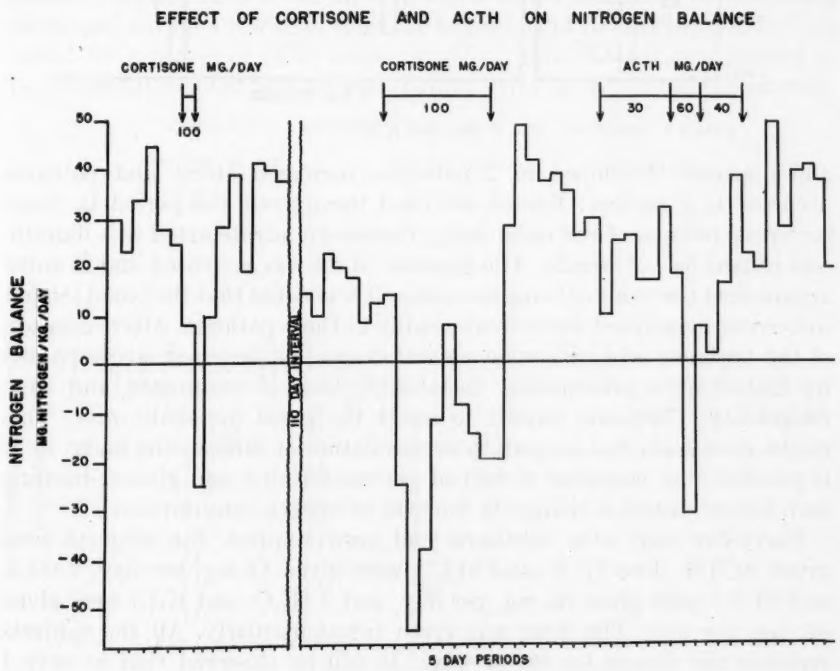

FIG. 5. (O.S.)

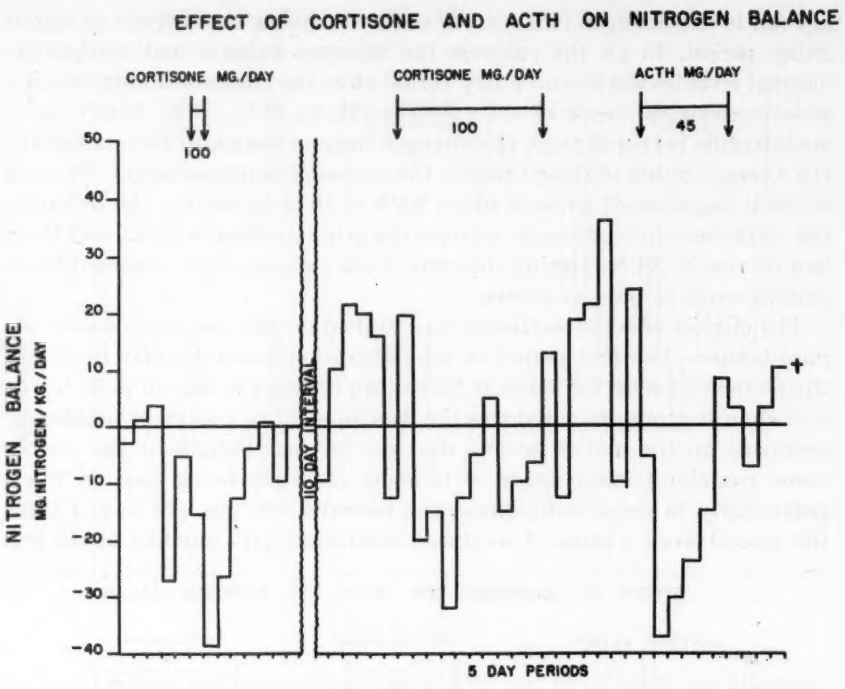

FIG. 6. (K.I.)

and dizziness developed in 2 patients. Increased thirst and polyuria occurred in 1 patient. Edema occurred throughout the period in those receiving 100 mg. of cortisone daily. Potassium nitrate acted as a diuretic and caused loss of weight. The appetite in all was increased and in some approached the point of being ravenous. It was noted that the blood tended to become hemolyzed much more readily in these patients. After cessation of the hormone administration mental depression occurred, accompanied by inability to concentrate, irritability, lack of endurance and easy fatigability. Cortisone tended to lower the basal metabolic rate. This might have been due in part to accumulation of fluid in the body, or it is possible that cortisone disturbed glucose-forming and glucose-burning and, hence, caused a change in the rate of oxygen consumption.

Forty-five days after cortisone had been stopped, the subjects were given ACTH. Two (D.E. and M.C.) were given 15 mg. per day, 2 (O.S. and M.A.) were given 30 mg. per day, and 2 (K.O. and K.I.) were given 45 mg. per day. The drug was given intramuscularly. All the subjects received the dosage for thirty days. It will be observed that in only 1 subject (K.I., 45 mg. daily) was there a marked fall in nitrogen balance

to negative values. This subject was also the one who showed closest to negative nitrogen balance without any medication. All the other subjects showed a positive balance under ACTH administration. With ACTH, only 1 subject (O.S.) showed an increase in blood NPN greater than 10 mg. per 100 cc. There was a decrease in nitrogen retention from its earlier high value obtained after the cortisone administration was stopped, but this decrease was not great and in most cases the trend to lower values was evident before the ACTH administration was started. After thirty days the ACTH was discontinued in 3 subjects (K.O., M.C., and K.I.). The other 3 subjects received an increased dosage, namely, 20 mg. intramuscularly three times daily for twelve days, then 20 mg. twice daily for eighteen days. It will be observed that a dosage of 60 mg. per day, produced a negative nitrogen balance in all 3 subjects.

Changes in the basal metabolic rate during the administration of ACTH were relatively insignificant. There was a tendency toward elevation in most instances. The maximum change in 1 individual (K.I.) was an elevation from −24 to −8 per cent. The glucose tolerance curves during the administration of ACTH tended to be of the flat variety. The clinical picture changed somewhat. In 4 of the 5 subjects a sense of well-being developed within a few days after the beginning of ACTH injections. This lasted for a period of three weeks, at which time the patients seemed to be somewhat more exhausted and slightly irritable. Edema of the extremi-

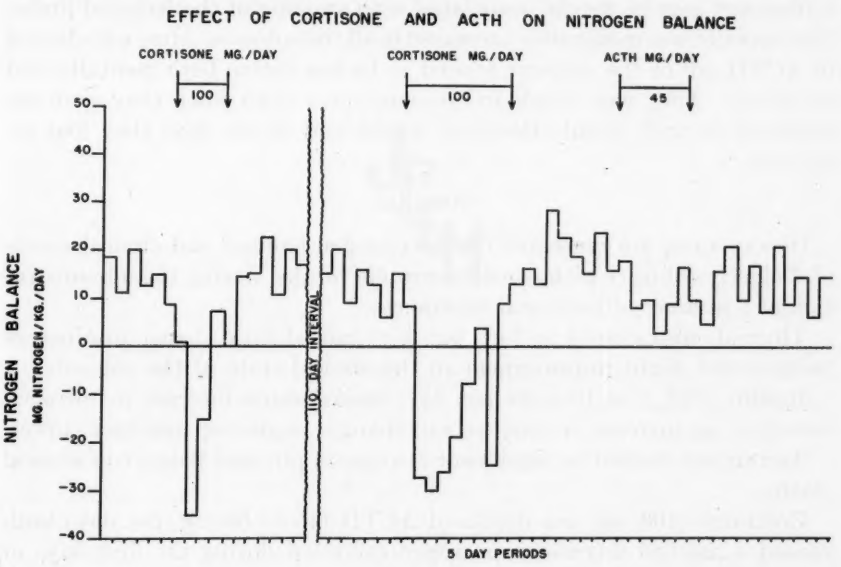

FIG. 7. (K.O.)

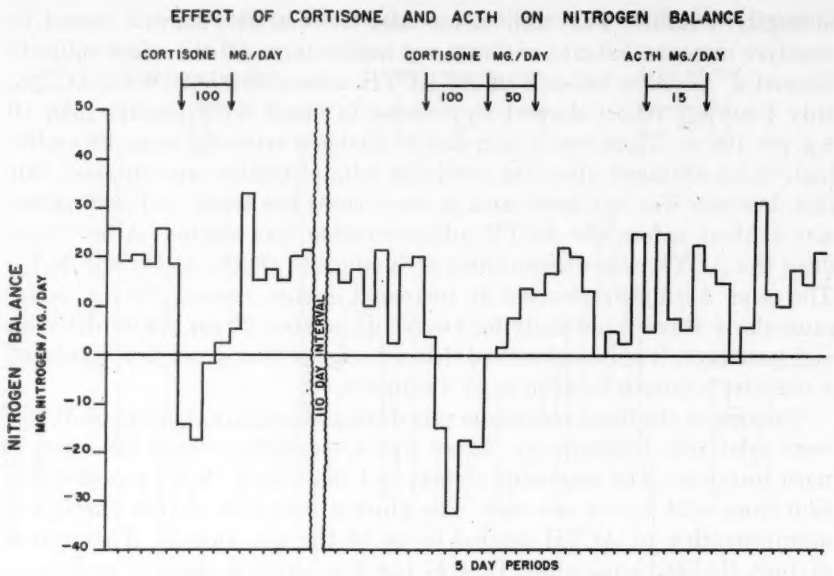

FIG. 8. (M.C.)

ties developed to a lesser extent than during cortisone therapy. There was a transient gain in weight, associated with swelling of the feet and limbs. The appetite was moderately increased in all the subjects. After withdrawal of ACTH, all of the subjects tended to be less active both mentally and physically. They were weak and became more tired when they were encouraged to walk about. However, at the end of ten days they had recovered.

SUMMARY

Observations are presented on the nitrogen balance and clinical status of 7 elderly subjects with constant protein intake, during the administration of a number of hormonal substances.

Thyroid substance ($\frac{1}{4}$ to 1 gr. per day) caused little change in nitrogen balance and slight improvement in the clinical state of the subjects.

Insulin (PZI, 5 or 10 units per day) caused some increase in nitrogen retention, an increase in appetite and changes in glucose tolerance curves.

Terramycin caused no significant changes in nitrogen balance or clinical status.

Cortisone (100 mg. per day) and ACTH (15 to 60 mg. per day) both caused a marked increase in nitrogen excretion during the first days of administration. There was a return to a positive nitrogen balance during

continued administration of either drug. There was some edema and water retention in all subjects. With both drugs initially there developed an increased feeling of well-being. After cessation of treatment, there was irritability and mental depression.

Acknowledgments

We wish to thank the following for their invaluable assistance in making these studies possible: Swift & Company, makers of Swift's Junior Meats; Merck & Co., makers of Cortone; The Armour Laboratories, makers of ACTH; Chas. Pfizer & Co., Inc., makers of Terramycin; and the Lambert Pharmacal Co., makers of Towsen-C.

We acknowledge, with thanks, the assistance of Mrs. Marjorie Tittsworth, R.N., who was in charge of the diets and the administration of all medication.

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FUNCTIONAL AND HISTOLOGIC EFFECTS OF THERAPEUTIC DOSES OF RADIOACTIVE IODINE ON THE THYROID OF MAN*

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THE purpose of this paper is to present observations on the effects of radiation in patients who have been treated with radioactive iodine. Attention has been directed toward: 1) the metabolism of the thyroid cells as measured by tracer doses of radioactive iodine before and after radiation therapy; and 2) the histologic features of cellular damage of the thyroid and the regenerative processes that may take place after large doses of radioactive iodine.

It has been adequately shown that a large dose of radioactive iodine impairs the functional capacity of the human thyroid (1–22). This principle has been employed in the treatment of some patients with Graves' disease (1–4, 6, 8, 9, 17–19, 21–35), others with functioning carcinomas of the thyroid (29–35) and still others with heart disease accompanied by normal thyroid function (17, 19, 28).

Although a large number of patients with thyrotoxicosis have been treated with radioactive iodine, relatively few observations have been published on the nature of the cellular damage and the regenerative processes that may follow. Seven cases reported in this series were previously discussed by Chapman and Evans (4, 44). Williams *et al.* (47) published photomicrographs of the thyroids of 2 patients who had been treated for Graves' disease with radioactive iodine. In 1 they reccgnized an increase in connective tissue but no detectable abnormality in the thyroid cells three months after the administration of 18 millicuries in divided doses. Shapiro (48) described marked distortion of the nuclei four weeks after a dose of only 2 millicuries of radioactive iodine. He also described

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"late radiation effects" one year after treatment and from this assumed that "the cells either undergo complete destruction and disappear or regenerate and regain their function."

Various degrees of radiation damage have been observed in experimental animals (5–7, 10–16, 36–39). However, in such instances, the driving force which causes Graves' disease was not present. If the driving force which induced the hyperplasia in Graves' disease in man remained operative after radioactive iodine therapy, the final status of the thyroid might be distinctly different from that in normal animals in which the stimulus to hyperplasia was not present. Therefore, the circumstances surrounding the recovery of the radiated thyroid in man suffering from Graves' disease and in a normal animal may be quite different.

The chief uncertainty in the use of radioactive isotopes is the possibility of the subsequent development of malignant changes in the irradiated tissues. The fear of this undesired effect of radiation has been present since the earliest therapeutic use of radioactive isotopes. Several authors (16, 49, 50, 51) have raised the question of the future development of cancer of the thyroid in patients given radioactive iodine. So far no reports of such an occurrence have appeared in the literature. In this study considerable importance is attributed to the opportunity to study human thyroidal tissue which had been subjected to large doses of radioactive iodine as long as eight years previously.

There are four main factors which influence the effect of radioactive iodine on the human thyroid. These are mass, energy, time, and the sensitivity of the cell to radiation.

1. The mass of the thyroid cannot always be accurately estimated by palpation in the living patient (25, 40); therefore, the amount of radiation delivered per gram of tissue may not be precisely known.

2. The amount of radioactive iodine collected by the thyroid and its total energy can be accurately determined, but the effect on the tissue may be materially altered by variations in distribution in the gland. In nodular goiters, it has been shown that the distribution varies from area to area (43). The importance of the distribution of the isotope is further emphasized by the fact that the beta rays from radioactive iodine have a destructive centrifugal spread of only about 2 millimeters.

3. The rate of uptake and release of the isotope by the thyroid cell varies with each patient.

4. The sensitivity of the thyroid cell to radiation probably varies with the degree of cellular function. A normally functioning cell is more resistant to roentgen radiation (41) than is a hyperplastic cell (42). Clinically this has recently been suggested by the reports (19, 28) that larger doses (microcuries per gram) of radioactive iodine are required to produce

myxedema in normal patients than in patients with diffusely hyperplastic goiters.

MATERIALS AND METHODS

During the past ten years, over 400 patients with thyrotoxicosis have been treated with radioactive iodine in the Thyroid Clinic of the Massachusetts General Hospital. In this series of patients the average treatment dose of I^{130} per patient was approximately 25 millicuries and the average treatment dose of I^{131} was 10 millicuries.[1] The clinical response of the earlier patients in this series has been previously reported (4, 18, 22, 44). In all patients the uptake of tracer doses of radioactive iodine was measured (46) before and, in most patients, at various intervals after the therapy had been given.

The samples of thyroid tissue were obtained from 25 patients from two days to eight years after therapeutic amounts of I^{130} or I^{131} had been given for hyperthyroidism. Tissue was also obtained from 4 patients who had been euthyroid at the time radioactive iodine therapy was given. The tissues were obtained: 1) by biopsy, in patients who were successfully treated; 2) by thyroidectomy, in patients with persistent hyperthyroidism; and 3) by necropsy, in patients who succumbed to some intercurrent disease. The tissues were fixed in Helly's solution and later the preparations were stained with hematoxylin and eosin and in some instances with Masson's trichrome stain; some tissues were used for the preparation of radioautographs.

RESULTS

Changes in uptake of radioactive iodine by the thyroid after radioactive iodine therapy, as measured by tracer doses

Coincident with the improvement in the clinical status of these treated patients, the uptake of I^{131} approached normal values.[2] This change occurred usually within two to five months; however, in a few patients the

[1] Some patients, upon whose thyroids histologic studies were made, had received radioactive iodine in the form of I^{130} (half-life, 12.6 hours) between 1941 and 1946 while this particular isotope was obtainable from the cyclotron at the Massachusetts Institute of Technology. Other patients, after 1946, received radioactive iodine in the form of I^{131} (half-life, 8 days) when this isotope became available from the Atomic Energy Commission. It should be kept in mind that tissue doses per hour from these two isotopes of iodine are different, as summarized by Evans (24). If the two isotopes, I^{130} and I^{131}, have the same initial dosage rates and if the isotopes are retained by the tissues, then I^{131} delivers a greater total dose of radiation than I^{130} because of its longer half-life. Although 1 millicurie of I^{130} per gram of tissue delivers 12.3 roentgen equivalents physical (r.e.p.) per minute, whereas I^{131} delivers 8.5 r.e.p. per minute, a proportionately larger dose of the short-lived isotope is required.

[2] In our experience, 20 to 55 per cent of a tracer dose of I^{131} is retained in the normal thyroid forty-eight hours after oral administration.

uptake continued to decline or did not reach a normal level until twelve or more months after therapy—long after detectable radioactivity had disappeared from the gland.

In 1 patient (#12, F.G., Table 1) the uptake of I^{131} declined from 85 per cent to 27 per cent in six weeks, but four months later it had increased to 51 per cent without concurrent clinical changes or a rise in the level of serum protein-bound iodine. It will be noted in the abstract of this patient that islands of apparently undamaged hyperplastic epithelium were subsequently found in the biopsy specimen of the thyroid. These islands may represent either regenerated tissue or tissue spared from radiation.

Observations suggest that maximal doses of radioactive iodine cause a relatively rapid lethal effect on the thyroid cells (such as in Patient #7, G.L., Table 1), resulting in hypothyroidism or myxedema in two or three months. Smaller doses may result in temporary damage or delayed death of cells; yet these cells have been able to collect sufficient amounts of a subsequent tracer of I^{131} to produce positive radioautographs (Patient #11, T.McL., Table 1). In some instances in which the dose of radioactive iodine had been insufficient to abolish the hyperthyroidism, the uptake remained above normal; but following additional treatment doses the uptake declined, coincident with the disappearance of hyperthyroidism. In contrast, Patient #20 (W.L., Table 1) received three treatment doses of I^{131} (14.8, 12.0 and 13.6 millicuries) without clinical improvement; the serum protein-bound iodine level remained elevated and the uptake of tracer doses of I^{131} remained above normal. At the time a subtotal thyroidectomy was performed in this case, relatively little destruction of the thyroid was found (Fig. 1). Observations on this patient suggested that there had been an unusually rapid uptake and release of the radioactive iodine by the thyroid.

Histologic changes in thyroid tissue resulting from large doses of radioactive iodine

The facts concerning the observations and treatment of 29 patients whose thyroids were subjected to histologic examination are summarized in Table 1. Some examples of the gross and microscopic anatomic details of the changes in the thyroids are presented here in the form of abstracts. It is important to point out that considerable variation in the histologic pattern may occur in the thyroids of patients with hyperthyroidism, especially in those glands containing nodules. A biopsy reveals only the changes in a small area of a gland, the pattern and function of which may be entirely different in other areas. Thus the interpretation of a single biopsy specimen must be made with considerable caution, because it may not be a true reflection of changes throughout the entire gland. The

TABLE 1. A SUMMARY OF FINDINGS IN PATIENTS FROM WHOM THYROID TISSUE WAS STUDIED FOLLOWING THERAPEUTIC DOSES OF RADIOACTIVE IODINE

Patient	Age (yrs.)	Sex	Pretreatment thyroid state, and av. BMR	Total dose of I^{131} or I^{130}* (millicuries)	Per cent of dose retained in 48 hrs.	Time between therap. dose and histologic study	Clinical change, and av. BMR	Histologic effect of radiation, graded + to ++++	Estimated wt. of thyroid (Gm.)	Estimated μc./Gm. of thyroid in 48 hrs.	Remarks
#1 R.W.	65	f	euthyroid (+10)	6	53	2 days	remained euthyroid	0	50	63	PBI 8.6γ% before I^{131} therapy.
#2 J.R.	53	m	euthyroid	25	79**	7 days	—	0	22	898	Cardiac failure; death due to coronary infarction; postmortem exam.
#3 J.C.	62	f	hyperthyroid (+86)	10	93	19 days	died in 19 days	+§	36	261	PBI 11.6γ% 4 days after I^{131} therapy; death from coronary occlusion; postmortem exam.
#4 L.B.	76	f	hyperthyroid (+13)	9	74	27 days	died in 27 days	+++	40	166	Died of coronary occlusion; postmortem exam.
#5 A.L.	47	f	hyperthyroid (+35)	14	98	40 days	died in 40 days (+44)	—	80	171	Died of heart failure and pneumonia; postmortem exam.
#6 F.D.	51	f	hyperthyroid (+39)	6.4	95	84 days	remained toxic	0	40	152	Thyroidectomy after 2 mos. preparation with propylthiouracil.
#7 G.L.		f	hyperthyroid (+11)	20	83	145 days	myxedema	++++	20	830	Died of heart failure; postmortem exam.
#8 N.S.	20	f	hyperthyroid (+40)	15	—	154 days	remained toxic	—	100	—	Subsequent thyroidectomy elsewhere after preparation with methylthiouracil.
#9 L.P.	15	f	hyperthyroid (+79)	7†	—	168 days	remained toxic (+40)	0	—	—	Died the day of thyroidectomy; propylthiouracil for 2 mos. preceding surgery.
#10 R.D.	55	f	hyperthyroid (+25)	11	83	170 days	remained toxic (+17)	0	60	125	Nodular goiter; subsequent thyroidectomy after 3 mos. of preparation with propylthiouracil.
#11 T.McL.	52	f	hyperthyroid (+45)	9	87	180 days	euthyroid in 2 mos.	++	60	115	Original PBI 16.0γ%; 25.5γ% 8 days post-treatment; 6.2γ% in 2 mos.; tracer uptake of I^{131} in 4 mos. was 54% and in 9 mos. 43%; biopsy.
#12 F.G.	48	f	hyperthyroid (+29)	7	97	256 days	euthyroid? (+18) in 4 mos.	+++	50	136	Original PBI 16.1γ%; methyl mercaptoidimazole 2 wks. before I^{131} therapy; PBI 6.5γ% in 6 wks. and 6.8γ% in 4 mos. after I^{131} therapy; tracer uptake 85% before I^{131} therapy, 27.2% in 6 wks., and 51% in 4 mos.; biopsy.
#13. E.W.	68	f	euthyroid	17	30	305 days	euthyroid	nodules spared	—	—	Thyroidectomy because of persistent nodularity.
#14. P.C.	44	m	hyperthyroid (+33)	15*	—	316 days	remained toxic	0	40	—	Thyroidectomy because of persistent thyrotoxicosis; KI started 3 days after I^{130} therapy.
				13.8*	—	121 days	remained toxic				
#15 C.D.Q.	21	f	hyperthyroid (+25)	10*	—	325 days	euthyroid in 1 yr.	0	40	—	BMR remained at pre-treatment level for 9 mos.; thyroidectomy for pressure symptoms.
#16 J.R.	26	f	hyperthyroid (+70)	10‡	—	328 days	remained toxic (+23)	++	—	—	Thyroidectomy after 2 mos. of preparation with mercaptoimidiazole.

* Millicuries of I^{130} so identified; otherwise I^{131} was given.

** Based on urinary excretion in a patient with renal insufficiency.

§ A definite distinction could not be drawn between early changes due to radiation and those due to postmortem autolysis in this gland obtained six hours after death.

† Treated by Dr. R. H. Williams.

‡ Treated by Dr. Saul Herts.

¶ While receiving potassium iodide.

Potassium iodide was given for a period after the treatment. BMR records are those obtained when no KI was being given.

¶ While receiving potassium iodide.

Potassium iodide was given for a period after the treatment. BMR records are those obtained when no KI was being given.

Patient	Age (yrs.)	Sex	Pretreatment thyroid state, and av. BMR	Total dose of I^{131} or I^{130}* (millicuries)	Per cent of dose retained in 48 hrs.	Time between therapy dose histologic study	Clinical change, and av. BMR	Histologic effect of radiation, graded + to ++++	Estimated wt. of thyroid (Gm.)	Estimated $\mu c./Gm.$ of thyroid in 48 hrs.	Remarks
#17 J.I.	50	m	hyperthyroid (+80)	40*	79	343 days	remained toxic (+67)		60	527	Pre-treatment biopsy; post-treatment biopsy.
				38*	95	297 days	remained toxic (+41)		60	601	
				48*	26	2 days	euthyroid (+6)	+++	45	277	
#18 W.B.	42	m	hyperthyroid (+30)	15*	—	1 yr., 1 mo.	remained toxic (+40)	0	—	—	Thyroidectomy after preparation with KI.
#19 R.P.	39		hyperthyroid (+40)	7	50	1 yr., 3 mos.	remained toxic (+25)	0	40	87	Toxic nodular goiter; hyperfunction in nodules; propylthiouracil for 2 wks. and biposy of one nodule before I^{131} therapy; tracer uptake 49% 4 mos.; PBI 10γ% 14 mos. after I^{131} therapy; thyroidectomy 14 mos. after I^{131} therapy.
#20 W.L.	45	f	hyperthyroid (+55)	14.8	90	1 yr., 4 mos.	remained toxic (+56)		120	105	Propylthiouracil for 5 mos. before I^{131} therapy; thyroidectomy after 8 mos. propylthiouracil preparation.
				12.0	91	1 yr., 3 mos.	remained toxic (+63)	+	110	109	
				13.6	66¶	209 days	remained toxic		80	112	
#21 F.B.	70	f	hyperthyroid (+30)	12	60	2 yrs., 7 mos.	euthyroid in 3 mos.	0	60	118	Tracer uptake 37%; PBI 8.9γ% 2 yrs. after I^{131} therapy; died of myocardial infarction; postmortem exam.
#22 E.D.	50	f	hyperthyroid (+27)	1.4*	—	2 yrs., 8 mos.	remained toxic (+40)	0	—	—	Nodular goiter; thyroidectomy after preparation with KI.
				1.8*	—	2 yrs., 7 mos.					
#23 M.L.	56	f	hyperthyroid	10	70	2 yrs., 11 mos.	remained toxic (+11)	+++	40	175	Propylthiouracil for 17 days before I^{131} therapy; 2 yrs. after I^{131} therapy PBI 11.8γ%; 2¼ yrs. after I^{131} therapy PBI 8.4 and tracer uptake 65%; biopsy.
#24 C.C.	25	f	euthyroid (+3)	40*	50	3 yrs.	no change (+6)	0	nodular goiter	—	Two nodules in thyroid; goiter considered to be reduced in size; subsequent thyroidectomy.
#25 M.S.	51	f	hyperthyroid (+36)	20*	66	4 yrs.	remained toxic (+29)		50	136	Died of widespread multiple myeloma; postmortem exam.
				10	81	3 yrs., 6 mos.	euthyroid in 2 mos.	++	55	146	
#26 A.M.	34	m	hyperthyroid (+23)	18*	—	3 yrs., 8 mos.	myxedema in 4 mos.	++++	40	—	Tracer of 1200 $\mu c.$ of I^{130} given 5 mos. before therapeutic I^{130} (BMR +42); biopsy.
#27 H.S.	49	f	hyperthyroid (+33)	20*	58	5 yrs., 4 mos.	remained toxic (+14)		40	290	Adenocarcinoma of the lung; patient died 2 mos. later; thyroid biopsy 2 mos. before death; postmortem exam.
				30*	38	5 yrs., 2 mos.	euthyroid in 1 mo.	+++	30	380	
#28 L.S.	42	m	hyperthyroid (+48)	43	82	6 yrs., 2 mos.	euthyroid in 1 yr.	+++	60	420	Biopsy.
#29 M.M.	38	m	hyperthyroid (+47)	15*	35	8 yrs.	remained toxic (+26)		120	42	Subtotal removal of right lobe because of the presence of a nodule 2 yrs. after first I^{130} therapy. Died of accidental causes; postmortem exam.
				53*	65	7 yrs., 10 mos.	remained toxic (+66)	+++	120	287	
				79*	50	7 yrs., 8 mos.	euthyroid in 1 mo.		70	565	

following abstracts are presented in a sequence based on the time which elapsed after radiation:

Examples of histologic changes in the thyroid of some patients treated with radioactive iodine and listed in Table 1

Patient #4, L.B.: 9 millicuries of I^{131} (74 per cent retained in forty-eight hours); Graves' disease accompanied by auricular fibrillation and cardiac failure. Propylthiouracil had previously been administered for three months. Death occurred from coronary occlusion twenty-seven days after I^{131} therapy. At postmortem examination the thyroid was slightly smaller than normal and contained no nodules.

There was diffuse accentuation of interfollicular connective tissue. Although occasional large acini containing colloid were encountered, small atrophic acini were most commonly found. These were composed of epithelial cells exhibiting oxyphilia and granu-

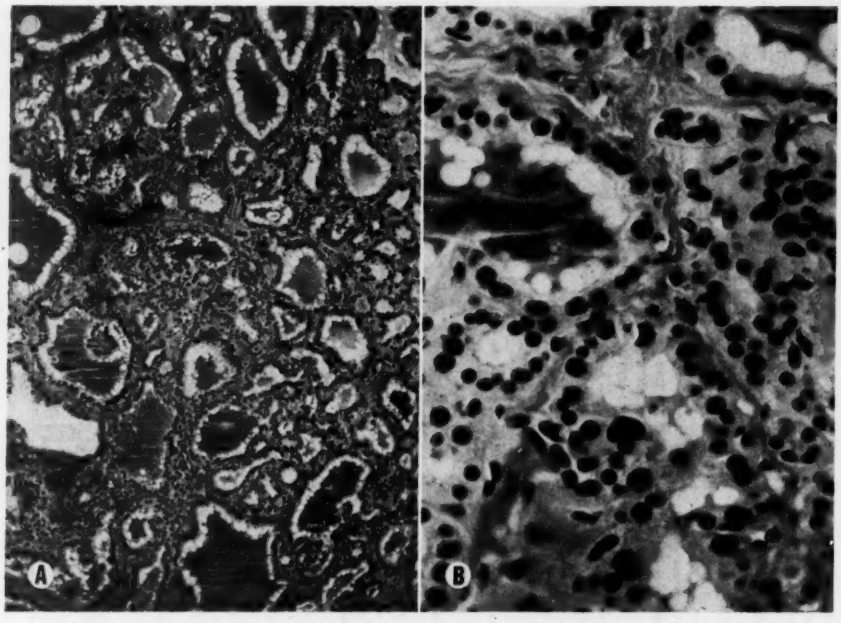

FIG. 1. Minimal destruction of the thyroid in a severely thyrotoxic patient who had received 40.4 millicuries of I^{131} in divided doses (*see* abstract, Patient #20, W.L.) A. Low-power view, showing persistent hyperplasia, in addition to a slight diffuse increase in interstitial connective tissue and nuclear hyperchromatism and pleomorphism. ×110. B. Higher magnification of a field in A. The atypicality in nuclear forms is prominent in an otherwise normal acinar pattern. ×600. (This patient received propylthiouracil following radioactive iodine treatment and preceding subtotal thyroidectomy. We have observed (39) in rats that nuclear atypicality following the radiation is accentuated by thiouracil compounds.)

larity of the cytoplasm. The nuclei varied from abnormally large, to small pyknotic and hyperchromatic forms (Fig. 2).

Patient # 7, G. L.: 20 millicuries of I^{131} (83 per cent retained in forty-eight hours); mild Graves' disease without enlargement of the thyroid and severe rheumatic heart disease with chronic congestive failure. Myxedema developed shortly before death, which was due to cardiac failure 145 days after treatment.

The thyroid was grossly fibrotic and adherent to surrounding structures. Microscopically, there was essentially total destruction of thyroidal architecture and replacement by dense fibrous tissue with widely scattered small empty spaces containing a few degenerated swollen epithelial cells, or a mass of colloid-like material (Fig. 3). Thrombosis was found in one small perithyroidal artery.

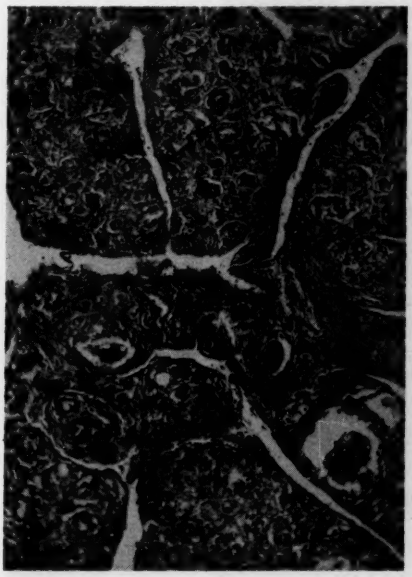

FIG. 2. The thyroid of a thyrotoxic patient who died of coronary occlusion twenty-seven days after treatment with radioactive iodine (*see* abstract, Patient #4, L.B.). ×150.

FIG. 3. The thyroid of a thyrotoxic patient who died of cardiac failure 145 days after treatment with radioactive iodine. Only a few acinar remnants are visible in the dense scar tissue (*see* abstract, Patient #7, G.L.). ×95.

Patient #11, T.McL.: 9 millicuries of I^{131} (87 per cent retained in forty-eight hours); moderately severe Graves' disease with diffuse enlargement of the thyroid. The patient became euthyroid within two months. A biopsy specimen was obtained 180 days after treatment.

There was an irregular increase in fibrous connective tissue in the stroma (Fig. 4). The follicles were generally small and contained atrophic thyroidal epithelium. Some follicles of medium size exhibited evidence of cellular hypertrophy and hyperplasia.

The colloid in the latter follicles was pale and scalloped at the periphery. The cytoplasm was conspicuously oxyphilic. Many nuclei were irregular in shape and the chromatin pattern was variable. Aggregates of lymphocytes appeared in areas where cellular damage was most noticeable. It was concluded that although there was a moderate degree of radiation damage, there were also focal areas (non-encapsulated) of hyperplasia.

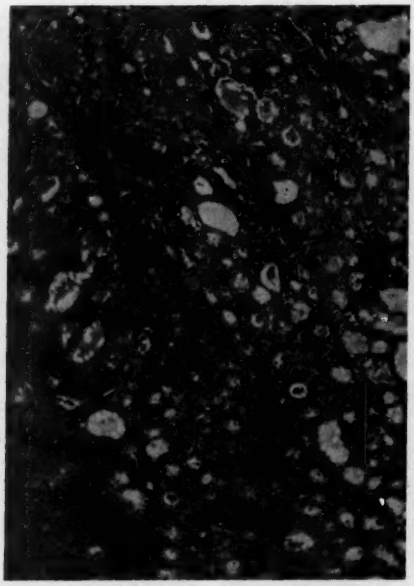

FIG. 4. The thyroid of a thyrotoxic patient successfully treated with radioactive iodine. Tissue was obtained 180 days after treatment (*see* abstract, Patient #11, T.McL.). ×110.

Patient #12, F.G.: 7 millicuries of I^{131} (97 per cent retained in forty-eight hours); Graves' disease with diffuse enlargement of the thyroid. The patient became euthyroid four months after treatment. At the time the biopsy specimen was obtained, 256 days after treatment, the thyroid was found to be approximately one-half normal size. Telangiectasia was conspicuous on the surface of the gland.

The general microscopic architecture of the gland was modified by prominent fibrous bands, which separated zones of parenchyma. The acini were generally small and in some instances without discernible lumens. Where colloid was present, it was sparse and pale. The nuclei ranged from large swollen forms with coarsely granular chromatin to small irregular hyperchromatic masses. Some follicles which appeared normal were scattered among others which showed the changes that were interpreted as radiation damage. Areas of hyperplastic tissue produced the greatest degree of blackening on a radioautograph.

Patient #13, E.W.: 17 millicuries of I^{131} (30 per cent retained in forty-eight hours). The available evidence suggests that this patient had a nodular goiter without hyper-

thyroidism at the time of radioactive iodine therapy. At the time of thyroidectomy, 305 days after treatment, the surface of the gland was covered with telangiectases and was moderately adherent to surrounding structures. The left lobe was composed of nodules covered by white leathery tissue.

There were extensive zones of fibrosis in which scattered isolated atrophic follicles were found. The damage to thyroidal epithelium in those areas was comparable to that in Patient #7, G.L. (Fig. 3). Several discrete encapsulated nodules were present. Here the follicles were medium to large and somewhat irregular in shape, as is often seen in an adenoma. In these nodules oxyphilia of the cytoplasm was prominent; nuclei were highly variable in size, shape, and chromatin pattern. The colloid was pale but fairly abundant. These changes varied considerably from nodule to nodule. This case probably illustrates the effect of radiation in a nodular goiter in which the extra-nodular tissue had been almost totally destroyed and the residual nodules showed variable changes, dependent upon differences in their uptake of radioactive iodine.

Patient #17, J.I.: 126 millicuries of I^{130} in divided doses (for uptakes of I^{131}, *see* Table 1); severe Graves' disease with diffuse enlargement of the thyroid. At the time a pretreatment biopsy specimen was obtained, the gland was red, meaty and extremely vascular.

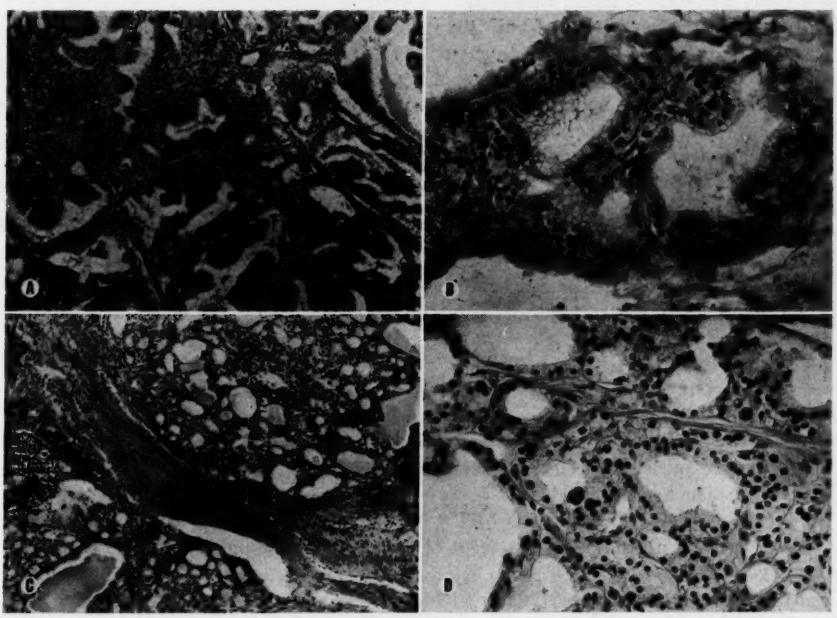

FIG. 5. Microscopic sections from pre- and post-radiation biopsy specimens of the thyroid in a thyrotoxic patient who was successfully treated with radioactive iodine in divided doses (*see* abstract, Patient #17, J.I.). A. Microscopic section from biopsy specimen of the thyroid prior to treatment with radioactive iodine. ×130. B. High-power magnification of A. ×430. C. Microscopic section from post-radiation biopsy specimen of the thyroid, 297 days after the second of three doses of radioactive iodine. ×130. D. High-power magnification of C. ×370.

The section from this biopsy specimen showed intense hyperplasia and cellular hypertrophy. The cells were uniformly very tall, having large nuclei which were located in the center, and cytoplasm which contained vacuoles at the apex (Fig. 5, A and B).

A post-radiation biopsy specimen was obtained 343 days after the first, and 297 days after the second of three doses of radioactive iodine (Fig. 5, C and D). Although at this time the patient still suffered from hyperthyroidism, considerable microscopic change was observed. Prominent coarse fibrous trabeculae divided the parenchyma. The follicles and the cells forming them were conspicuously smaller than formerly; however, the height of the cells was not entirely uniform. At least some nuclei in each follicle were atypical in size and shape, varying from shrunken hyperchromatic forms to those two or three times the normal diameter. The colloid in the follicles was depleted. In spite of these changes, considered to be radiation damage, the tissue contained some evidence of hyperplasia.

Patient #19, R.P.: 7 millicuries of I^{131} (50 per cent retained in forty-eight hours). This patient had thyrotoxicosis and several firm discrete nodules in the thyroid, one of which subsequently proved to be carcinoma.

A biopsy of one of the nodules in the right lobe before treatment revealed (by radioautograph) the presence of a hyperfunctioning nodule which histologically was classified as a fairly well differentiated adenoma.

Because of the persistence of both thyrotoxicosis and the nodule in the left lobe one year and three months after treatment with radioactive iodine, a thyroidectomy was performed. Neither gross nor histologic damage was observed in the thyroid. The nodule, which a radioautograph had indicated to be hyperfunctioning at the time of the previous biopsy, was still hyperfunctioning. The extranodular tissue was moderately atrophic and took up little radioactive iodine (Fig. 6, A and B), as is usually found in patients with thyrotoxicosis caused by hyperfunctioning nodules (43). The nodule previously noted in the left lobe was found to be papillary adenocarcinoma.

Patient #20, W.L.: 40.4 millicuries of I^{131} in divided doses (for uptakes of I^{131}, *see* Table 1); severe Graves' disease with diffuse enlargement of the thyroid. One year and four months after I^{131} treatment, thyrotoxicosis persisted. Following preparation with propylthiouracil, a bilateral subtotal thyroidectomy was performed. Grossly the gland was firm and grayish.

Slight diffuse interstitial fibrosis was present throughout the gland. The follicles were medium in size and showed evidence of hyperplasia (Fig. 1A). The thyroidal epithelium was cuboidal, and the colloid was vacuolated adjacent to the epithelium. The nuclei were variable in size and in some areas were hyperchromatic (Fig. 1B). Despite these atypical nuclei the cells were evidently viable and functioning, because severe hyperthyroidism had persisted.

Patient #28, L.S.: 43 millicuries of I^{130} (82 per cent retained in forty-eight hours); Graves' disease with diffuse enlargement of the thyroid. The patient became euthyroid in one year.

At the time the biopsy specimen was obtained, six years and two months after treatment, the thyroid was less than normal in size. The surface was pale and covered with telangiectases. A gritty sensation was encountered when the gland was cut. The sample of tissue showed diffuse fibrosis with islands of thyroid parenchyma consisting of distorted follicles with very pale colloid (Fig. 7, A and B). The thyroidal epithelium was variable in height. Almost all nuclei were irregular in shape and contained a wide variety of chromatin patterns (Figure 7C). Although extremely bizarre nuclear forms were present, none could be definitely regarded as mitotic.

Patient #29, M.M.: 147 millicuries of I^{130}, in divided doses (for uptakes of I^{130} *see* Table 1); Graves' disease with diffuse enlargement of the thyroid. The patient had previously undergone a subtotal thyroidectomy. The right lobe of the thyroid was resected 630 days after radioactive iodine treatment, because a 2-cm. nodule had developed in this lobe.

At the time of the operation the gland was found to be enlarged, very firm, and adherent to surrounding structures. Microscopic examination revealed shrunken atrophic remnants of follicles occurring as islands in dense connective tissue. Only minute amounts of colloid remained in the follicles. The cells varied markedly in size, and contained nuclei which were hyperchromatic and decidedly irregular in shape. At the time of this patient's sudden death (gun shot) eight years after radioactive iodine treatment, the changes were essentially the same as described above. The bizarre nuclear forms remained.

It became apparent early in the histologic appraisal of the material from various patients that there was a wide range of tissue response to the radiation from radioactive iodine. The thyroids of 10 of the 25 thyrotoxic patients showed no histologic changes attributed to radiation. In contrast, the remaining thyroids exhibited various degrees of histologic change rang-

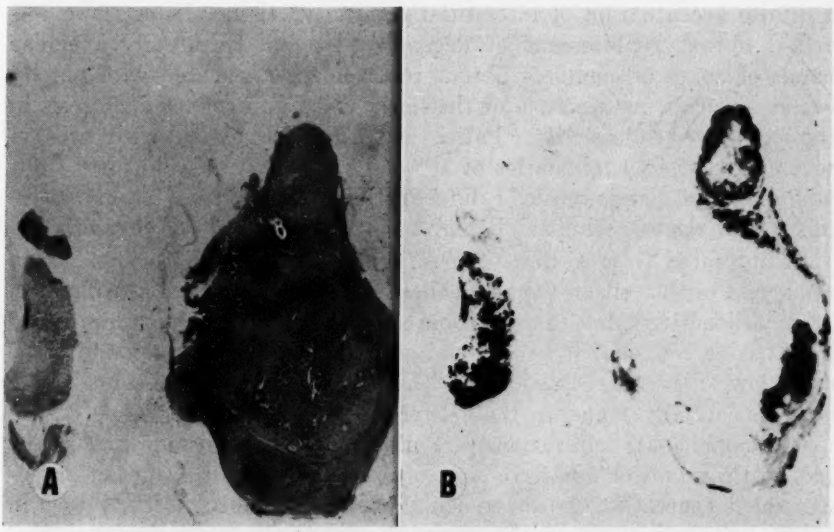

FIG. 6. The thyroid of a patient with nodular goiter and hyperthyroidism treated with radioactive iodine (*see* abstract, Patient #19, R.P.). A. Microscopic section of the entire thyroid, one year and three months after radioactive iodine therapy. The gland contained several hyperfunctioning nodules and a single nodule of papillary adenocarcinoma. The nodule containing carcinoma was palpable but its nature was not suspected before radioactive iodine was given. B. Radioautograph prepared from tissue section in A. The hyperfunctioning nodules caused marked blackening on the film, in spite of previous therapy. The nodule which produced no blackening consisted of papillary adenocarcinoma.

ing from minor alterations to extensive destruction with replacement by dense scar tissue.

The most outstanding alterations in the thyroid cells attributable to the radiation were found in the nuclei. Such changes consisted of irregularities in size and outline and were often associated with bizarre distortion of the chromatin pattern (Figs. 1B, 5D, and 7C). These bizarre nuclear forms exhibited eccentric or retort-like outlines and homogeneously dense chromatin; they often varied considerably from acinus to acinus, and occasionally abnormal nuclei alternated with morphologically normal nuclei within the same acinus. Nuclear abnormalities were commonly associated with evidences of cytoplasmic damage, such as eosinophilia, granularity, vacuolation and loss of cellular membranes. The cytoplasmic alterations, although often quite impressive, were generally less constant and specific than the nuclear changes.

Fibrosis within the parenchyma of the thyroid was a rather consistent sequela of radioactive iodine therapy. This fibrosis appeared to be initially a diffuse accentuation of interstitial connective tissue. Later there was actual fibrous replacement of degenerated acini. Eventually irregular bands or zones of complete fibrous replacement alternated with patchy islands of acini. An example of the fairly rapid appearance of fibrosis in the thyroid was observed in Patient #4, L.B. This patient died twenty-seven days after 9 millicuries of I^{131} (74 per cent uptake in forty-eight hours) and the gland revealed a diffuse increase in the fibrous stroma, intermixed with degenerating acini (Fig. 2).

In untreated Graves' disease there is a relatively uniform increase in the height of the cells in the acini; these have been described as columnar. After radioactive iodine therapy some cells remained columnar while others in the same acinus or in other acini of the same gland were found to be quite low (Figs. 5B, 5D and 7C). This irregularity in the height of the cells was usually found in those thyroids which showed bizarre nuclei.

Occasional focal infiltrations of lymphocytes were encountered in the radiated thyroids. These were most often associated with zones of parenchymal degeneration; however, the common finding of lymphocytes in the thyroids of patients with Graves' disease modifies the significance of their presence. Acute inflammatory cellular reactions were not observed in thyroidal tissue from any patient. This may have been due to the fact that the tissue was usually obtained relatively late in the post-radiation period. Studies made by others (12) on rats have revealed acute tissue responses with areas of polymorphonuclear infiltration and focal necrosis as early as twelve to twenty-four hours after the administration of relatively large doses of radioactive iodine.

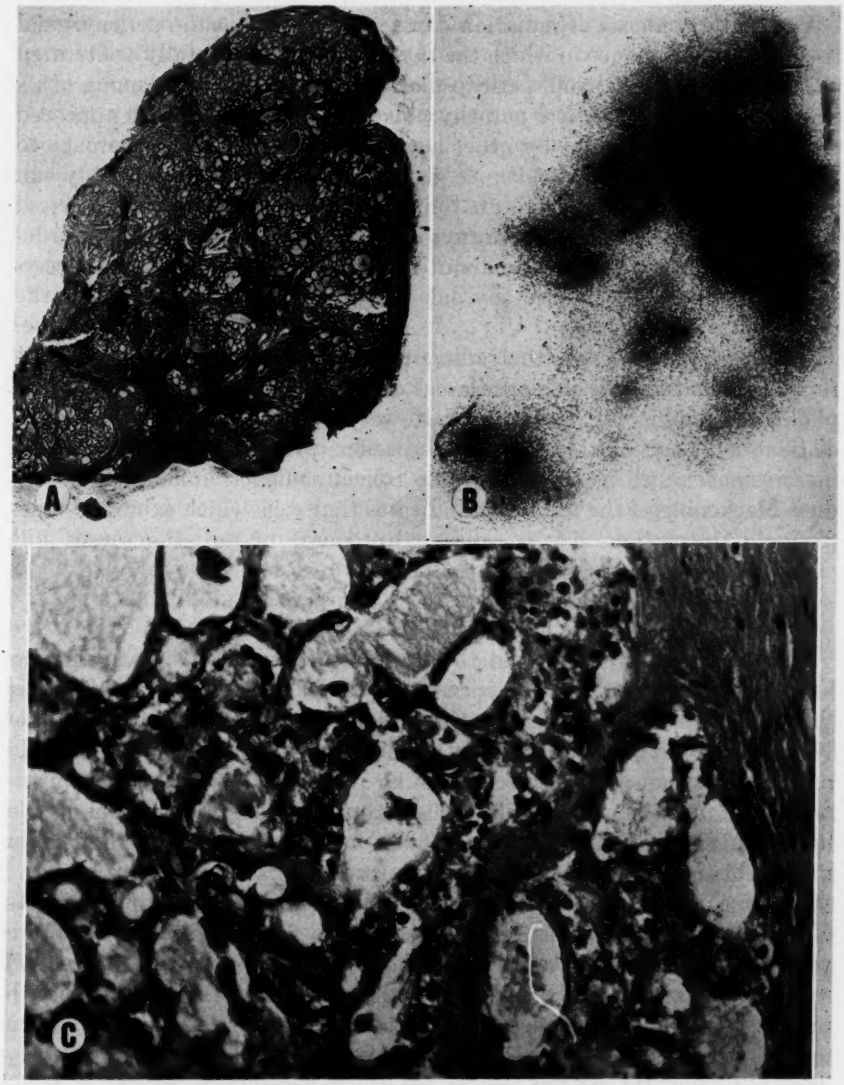

FIG. 7. The thyroid of a previously thyrotoxic patient successfuly treated with radioactive iodine, six years and two months before biopsy of the thyroid. A tracer dose of I^{131} was given prior to the procurement of the biopsy specimen (*see* abstract, Patient #28, L.S.). A. Microscopic tissue section of the thyroid, survey view. ×25. B. Radioautograph prepared from A. Areas of blackening represent the location of the radioactivity in the tissue. ×25. C. High-power magnification of a functioning area in A. Note the atypical nuclear forms. ×350.

Very little evidence of radiation damage was found in the perithyroidal tissues. In one instance in which the thyroid was almost totally obliterated (Patient #7, G.L.), a small pericapsular artery exhibited thickening of its wall and thrombosis. A few parathyroids were examined and all appeared normal. The descriptions by other authors (10, 12, 14) of the damage to the parathyroids of rats and mice given radioactive iodine probably can be explained by the intimate anatomic relationship between the thyroid and parathyroid. Since the beta rays of radioactive iodine have a destructive centrifugal spread of only about 2 millimeters in tissue, the parathyroid often imbedded in the thyroid of a rat is most vulnerable to the radiation.

As might be expected, the radioautographs prepared from 6 patients revealed that the least damaged areas caused the most blackening on the photographic film (Fig. 7, A and B). However, other areas which displayed distinct evidence of the effect of radiation, principally in the form of bizarre nuclei, still had a capacity to collect radioactive iodine and produce blackening of the film. This indicates that cells which exhibited morphologic alternations due to sublethal amounts of radiation, were still capable of producing thyroid hormone.

DISCUSSION

It has been pointed out that the thyroids of 10 of the 25 patients who had been treated for thyrotoxicosis with radioactive iodine in this series showed no histologic changes attributable to the radiation. Most of these 10 patients had persistent hyperthyroidism. There are several possible reasons to explain the absence of cellular response: 1) an inadequate concentration of radioactive iodine per gram of thyroid; 2) variations in the functional state of the thyroid; and 3) an inability on our part to identify minute histologic changes produced by radiation.

In some of the thyroids, islands of tissue appeared devoid of the effects of radiation, especially in those patients with Graves' disease who were not rendered euthyroid by the radioactive iodine therapy. Gorbman (7) reported foci of relatively undamaged thyroid tissue in mice treated with radioactive iodine. He observed some of his animals as long as eighteen months, with no change in the appearance of the "surviving" partially damaged acini. He did not find neoplastic or metaplastic growth in these "surviving thyroid elements" and he stated that it seemed "quite obvious that surviving thyroid elements are incapable of regeneration."

There are several possible explanations for the finding of acini composed of altered or unaltered cells in a radiated thyroid: 1) tissue spared from radiation; 2) regenerated tissue, occurring in the post-radiation phase; 3) tissue partially damaged by radiation but surviving in this form; and 4)

as will be mentioned later, cellular mutations which have been propagated from cells that were partially damaged.

Goldberg and Chaikoff (16) found adenomas in the thyroids of 2 of 10 rats eighteen months after radioactive iodine had been given, but stated "that these did not appear malignant"; however, in the same publication these authors raised the possibility that the adenomas might be malignant on the basis of morphologic criteria. They also mentioned that Moon (52) found 6 adenomas in 14 presumably normal rats of the same strain. In a very recent publication Goldberg and Chaikoff (53) have presented further evidence for the carcinogenic effect of I^{131} in the thyroid of the rat. They found carcinoma of the thyroid in 7 of 25 rats (5 with distant metastases) one and a half to two years after a single dose of 400 microcuries of I^{131}. Doniach (54) administered radioactive iodine, methylthiouracil, acetylaminofluorene, alone, and in combination to rats. He reported that the formation of thyroid adenomas was significantly increased in animals treated with radioactive iodine and a goitrogen. The significance of these observations is somewhat modified by the fact that Griesbach *et al.* (55) and Money and Rawson (56) have produced such tumors with goitrogens alone. On the other hand, in experiments on rats some of us (39) have observed that thiouracil accentuates the bizarre nuclear changes produced by large doses of radioactive iodine, or brings into evidence such changes which are latent in animals that have received small doses of radioactive iodine. In the light of the latter observations, it may be that the administration of a thiouracil compound, in at least one of these patients who was subsequently subjected to thyroidectomy, may have accentuated some of the nuclear changes found (Patient #20, W. L.; Fig. 1, A and B).

The finding of abnormal thyroid cells exhibiting the effects of radiation as long as eight years following radioactive iodine therapy (Patient #29, M.M.) raises an interesting question. Although the duration of life of the normal thyroid cells is unknown, these apparently damaged cells have either survived eight years or represent propagations as mutated forms. If it is postulated that partially damaged or even normal cells do not survive eight years, then consideration must be given to the theory of the development of mutants. Answers to these questions are at present in the speculative stage but their importance relative to the possibility of potential neoplasia is obvious.

No case reports have been found in the literature describing carcinoma of the thyroid resulting from radioactive iodine. Two cases reported in this present analysis were found to have malignant disease in the thyroid; however, in 1 case (Patient #19, R.P.) the same nodule which subsequently was found to consist of papillary adenocarcinoma had been palpable at the time that the radioactive iodine was given, and in the second case

(Patient #25, M.S.) death was due to widespread multiple myeloma, one lesion of which was found in the thyroid.

SUMMARY

A study has been made of the functional and histologic changes in the human thyroid following therapeutic doses of radioactive iodine. After successful radioactive iodine therapy for Graves' disease, the uptake of tracer doses of I^{131} approached the values found in normal persons. In some patients this function declined during a period of twelve months or more, indicating a continuation of functional change in the thyroid cells long after disappearance of detectable radioactivity in the thyroid area.

Thyroid tissue was obtained from 29 patients by thyroidectomy, biopsy or autopsy, from two days to eight years after treatment with radioactive iodine. Anatomic changes, such as gross atrophy, telangiectases on the surface of the gland and adherence to adjacent structures were found in some of these thyroids.

The chief microscopic changes attributable to this radiation were irregularity in size and outline of the nuclei and bizarre distortion of the chromatin patterns. Fibrosis associated with groups of acini, containing cells of variable height, seemed to be a characteristic reaction to radiation injury. Extrathyroidal tissue appeared to have escaped injury from radioactive iodine.

Radioautographs of these thyroidal tissues disclosed that certain acini, although exhibiting bizarre nuclei, had a capacity to collect subsequent tracer doses of the isotope. Such areas of sublethal radiation injury probably are capable of producing thyroid hormone.

In a series of over 400 patients treated with radioactive iodine at the Massachusetts General Hospital during the past ten years no known carcinoma of the thyroid attributable to this agent has developed. Definite answers to the question of carcinoma formation must await prolonged observation of treated patients.

Acknowledgment

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SEPARATION OF 17-KETOSTEROIDS IN A 24-HOUR URINE SAMPLE

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INTRODUCTION

CALLOW and Callow (1) reported the separation of androsterone, dehydroepiandrosterone, and etiocholane-3α-ol-17-one by a chromatographic technique in which aluminum oxide was the adsorbent and a carbon tetrachloride-ethanol system was used for elution. Dobriner and his co-workers (2, 3, 4) extended the use of aluminum oxide for steroid chromatography and also used magnesium silicate, obtaining separation of 42 different urinary steroids by means of repeated chromatographing of extracts from large amounts of urine. The isolated compounds were characterized by melting-points and by infrared analysis. Dingemanse, Huis in't Veld, and de Laat (5) published a method for the determination of the individual 17-ketosteroids in a 24-hour urine by a single chromatographic process, using alumina as adsorbent, and benzene and benzene-ethanol mixtures for elution. The 17-ketosteroid content of each fraction was determined by the Zimmermann reaction, and the 17-ketosteroids were identified by 1) their position in the chromatogram, 2) addition of pure steroids to urines of known composition, or 3) isolation of crystalline steroids from fractions pooled from several chromatograms. A ratio of 2,500 parts of adsorbent to 1 part of 17-ketosteroid was used, and 48 fractions of 50 ml. each were collected. This method has been used by other workers, including Robinson and Goulden (6) and Devis (7). Microscale adaptations have been devised by Zygmuntowicz *et al.* (8) and by Pond (9).

Silica gel was used as the adsorbent in the study reported in this paper because it is a mild adsorbent which would bring about minimal changes in the steroid molecule. The urinary steroids were identified in the following ways: 1) by analogy with crystalline materials known to be eluted under the same conditions in the standard chromatogram, 2) by infra-

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red analysis of materials pooled from successive chromatograms, and 3) by paper chromatographic techniques.

METHODS

1. *Preparation of urine extracts for chromatography*

The 24-hour urine collections were boiled for eight minutes with 15 volumes per cent of HCl to hydrolyze the steroid conjugates, rapidly cooled, and extracted with ether. The total neutral fraction of the ether extract was obtained by the method of Pincus (10) and the ketonic fraction of the total neutral steroids was obtained through the use of the Girard reagent (11). The ketonic fraction was subjected to digitonin precipitation according to the procedure of Butt, Henly and Morris (12). The digitonin-nonprecipitable fraction, called the alpha-ketonic fraction hereafter, was then chromatographed. All 17-ketosteroid values were determined by the Zimmermann reaction in the manner previously described by Pincus (10).

2. *Silica-gel chromatographic procedure*

The columns used for chromatography consisted of a 50-ml. bulb, with a side arm and a long neck (4.5 cm.), attached to Pyrex tubing approximately 19 cm. in length and 1 cm. in inside diameter. To this was attached a section about 2 cm. in length and 0.5 cm. in inside diameter, which was joined to a No. 2 stopcock. A plug of glass wool was inserted at the point where the column narrowed. Silica gel (Davison Chemical Corp.; mesh size, thru 200) was weighed rapidly into a small beaker (the amount of silica gel used will be discussed in the Experimental Section) and immediately wet with a mixture of 1 part of anhydrous benzene (Merck, Reagent Grade, or Baker's Analyzed) and 3 parts of petroleum ether (Mallinckrodt Chemical Co., redistilled). The column was prepared as a slurry, using a total of 100 cc. of the 1:3 benzene-petroleum ether mixture (Fraction 1). Positive nitrogen pressure was applied through the side arm during packing, the neck of the bulb being stopped with a rubber stopper covered with aluminum foil. A plug of glass wool was placed on top of the column before the addition of the urine extract. After the addition of the urine extract, the nitrogen pressure was regulated so that the time for collecting 40 ml. of eluate was about five to eight minutes. With more polar solvent mixtures, this rate could not be exceeded without causing the stopper to be pushed from the neck of the bulb.

The alpha-ketonic urine fractions to be chromatographed were stored in a desiccator over $CaCl_2$ until immediately before they were placed on the column. They were then dissolved in 25 ml. of benzene, and 75 ml. of petroleum ether was added at the last minute, to minimize precipitation. Aliquots for colorimetry were removed and an aliquot of the solution estimated to contain from 4 to 8 mg. of 17-ketosteroid (by Zimmermann) was poured into the column. Fraction 2 consisted of this aliquot volume plus the first of a series of five 40-ml. portions of 1:3 benzene-petroleum ether. The standard chromatographic elution procedure finally adopted is shown in Table 2. Redistilled anhydrous ether (Mallinckrodt, Analytical Reagent) was used in the ether-benzene fractions. One hundred and forty-five fractions were collected, over several working days. The column was tightly stoppered overnight; it was never left overnight while being eluted with petroleum ether mixtures or 1:1 ether-benzene.

The eluates were collected in 50-ml. wide-mouthed Florence flasks and evaporated to dryness on a steam bath. They were made up to 10 cc. with 95 per cent ethanol and 1-ml. and 4-ml. aliquots were taken for the Zimmermann reaction. If the amount of color

in the 4-ml. aliquot was insignificant, the fraction was considered not to contain any significant amount of 17-ketosteroid. The micrograms of 17-ketosteroid in the fraction were plotted against the fraction number, and the amount of material in each peak calculated and reported as the percentage it represented of the total 17-ketosteroid put on the column. Because so many aliquots were removed for 17-ketosteroid analysis during the preparation of the extracts for chromatography, and for comparison between urine extracts, this was considered preferable to reporting the various components in absolute amounts, though a calculation of the absolute amounts in a 24-hour urine could easily be made from the percentage distribution.

3. *Infrared analysis*

Carbon disulfide solutions of various urinary fractions were studied in a 12C Perkin-Elmer infrared spectrometer, over a range between 2.5 and 15 μ. Tracings were automatically recorded and compared with those of crystalline steroids.

4. *Paper chromatographic techniques*

The paper chromatographic techniques used were the ligroin-propylene glycol system of Savard (13), a modification of this using heptane instead of ligroin, and the heptane-phenylcellosolve system of Neher and Wettstein (14). Varying time intervals were used, depending upon the peaks being resolved (the details are discussed with the results). The individual components were visualized by developing the Zimmermann color directly on the paper strips. Approximately 10 μg. of 17-ketosteroid in a single zone on a 1-cm. strip can be visualized by this reaction.

EXPERIMENTAL

1. *Standardization of chromatographic procedure*

In preliminary studies with pure crystalline steroids, using a ratio of 200 parts of silica gel to 1 part of steroid, it was found that the steroids were eluted reproducibly. The order of elution, and solvent mixtures for elution are shown in Table 1. When a mixture of androsterone, etiocholane-3α-ol-17-one, $\Delta^{2 \text{ or } 3}$-androstene-17-one, and 11-hydroxyandrosterone was chromatographed, four peaks were obtained. Figure 1 shows the degree of

TABLE 1. ORDER OF ELUTION OF CRYSTALLINE 17-KETOSTEROIDS FROM SILICA GEL

$$\left\{\frac{\text{Wt. silica gel}}{\text{Wt. steroid}} = \frac{200}{1}\right\}$$

Steroid	Eluted with	
$\Delta^{2 \text{ or } 3}$-androstene-17-one	benzene—pet. ether	1:1
3β-chloro-Δ^5-androstene-17-one	benzene—pet. ether	1:1
Pregnane-3α-ol-20-one	ether—benzene	1:49
Androsterone	ether—benzene	1:49
Etiocholane-3α-ol-17-one	ether—benzene	1:49
11β-hydroxyandrosterone	ether—benzene	1:4

resolution. The amounts of material in the peaks corresponded with the amounts of the individual steroids put on the column. Androsterone and etiocholane-3α-ol-17-one were not completely separated, but an accuracy of 10 per cent in estimation was obtained when the minimum point was considered to indicate the end of the elution of androsterone and the beginning of the elution of etiocholane-3α-ol-17-one.

At this time, because the resolution of urinary components on 200:1 silica gel was not satisfactory, a change was made to a ratio of 1,000 parts

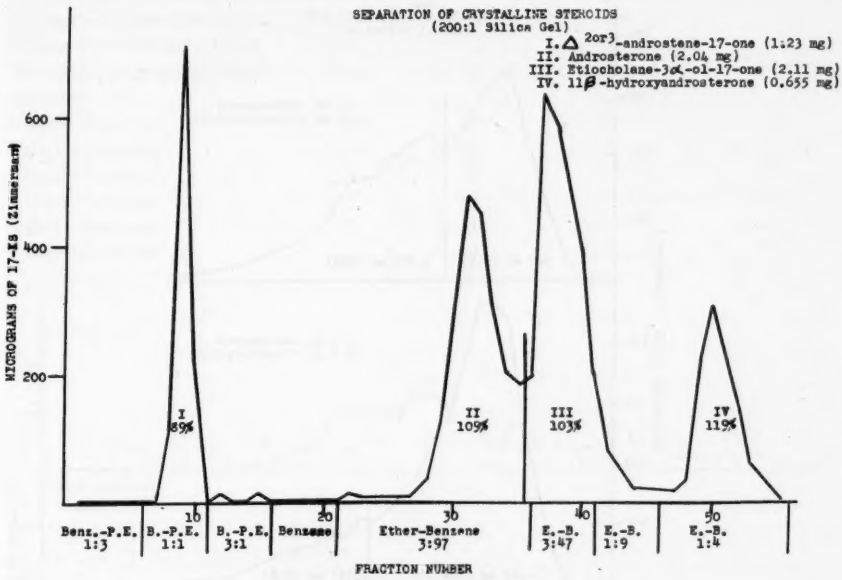

FIG. 1. Separation of crystalline steroids.

of silica gel to 1 part of 17-ketosteroid. Figure 2 shows chromatograms of a 1:1 mixture and a 4:1 mixture of androsterone and etiocholane-3α-ol-17-one on 1,000:1 silica gel. Elution with 1:19 ether-benzene gave partial separation into two peaks. Estimation of the amounts of each compound, again using the point at which the secondary rise begins as the boundary between the androsterone and etiocholane-3α-ol-17-one elutions, gave recoveries of 94 and 96 per cent of each compound when the ratio was 1:1. The separation was far from satisfactory when the ratio was 4:1, although estimation from the point indicated on Figure 2 gave 101 and 90 per cent recovery of each compound.

When 1,000:1 silica gel was used, a lag in the appearance of all peaks

was seen. Preliminary studies, chromatographing alpha-ketonic urinary fractions on 200:1 silica gel, led to the appearance of seven characteristic peaks. The first of these was eluted with 1:1 benzene-petroleum ether. When 1,000:1 silica gel was used, this first peak did not appear until eluted with 3:1 benzene-petroleum ether and benzene, and corresponding delays were seen throughout the chromatogram. The system of chromatography finally set up as standard in this laboratory is shown in Table 2, and described in the section on Methods.

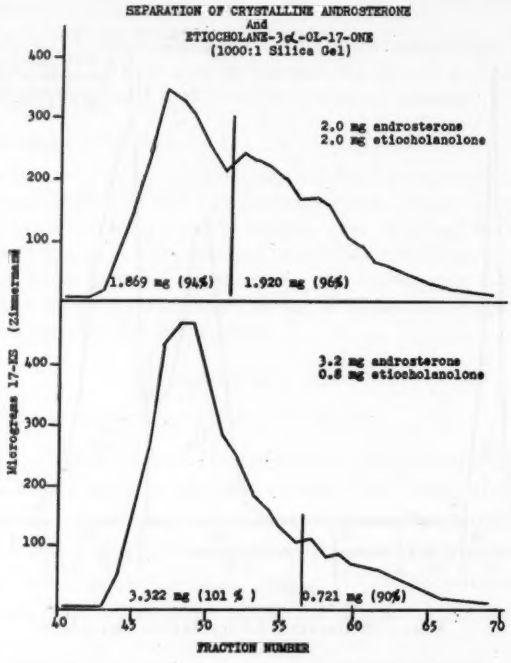

FIG. 2. Separation of crystalline androsterone and etiocholane-3α-ol-17-one.

2. *Chromatography of neutral α-ketonic urine extracts*

The standard chromatographic procedure was applied to α-17-ketosteroid extracts of urines of 12 normal young men, between 20 and 36 years old. Nine peaks were observed.

Figure 3 shows 3 urinary chromatograms. Resolution of androsterone (IV) and etiocholane-3α-ol-17-one (V) varied in completeness. In a series of 33 chromatograms of urines from schizophrenic as well as from normal young men, the resolution of the two compounds (referring to the types in Figure 3) was "good" in 12 (36 per cent), "average" in 15 (45 per cent), and "bad" in 6 (18 per cent).

TABLE 2. STANDARD CHROMATOGRAPHIC PROCEDURE FOR α-KETONIC EXTRACTS OF URINE

$$\left\{\frac{\text{Wt. silica gel}}{\text{17-KS (Zimmermann)}} = \frac{1000}{1}\right\}$$

Eluent mixture		Number of fractions (40 ml./ fraction)	Fraction No.	Peaks obtained from urine extracts
Benzene—petroleum ether	1:3	5	1– 6	
Benzene—petroleum ether	1:1	5	7– 11	
Benzene—petroleum ether	3:1	5	12– 16	I
Benzene		5	17– 21	
Ether—benzene	1:49	20	22– 41	II
Ether—benzene	1:19	44	42– 85	III, IV, V
Ether—benzene	1:9	10	86– 95	VI
Ether—benzene	3:17	10	96–105	VII
Ether—benzene	1:4	21	106–126	VIII
Ether—benzene	1:1	19	127–145	IX

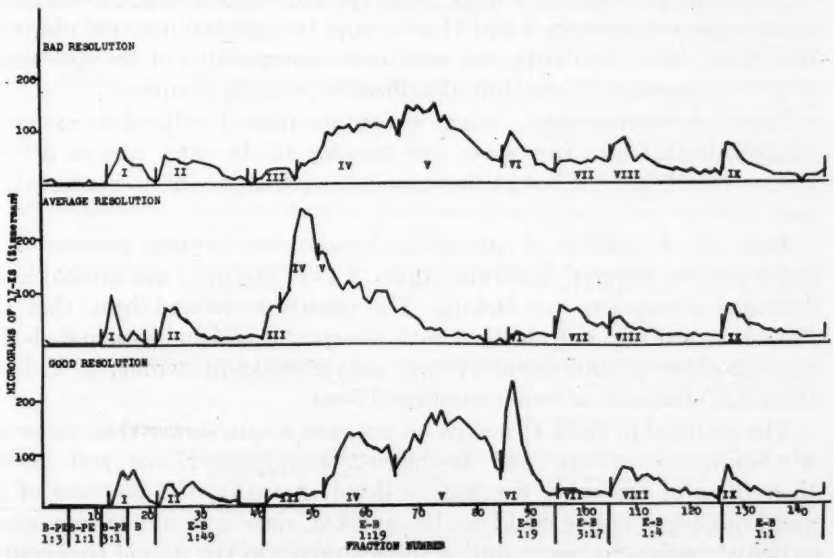

FIG. 3. Resolution of urinary 17-ketosteroids*

* The terms "good," "average" and "bad" refer to the resolution of androsterone (IV) and etiocholane-3α-ol-17-one (V). "Good" resolution was obtained in 36 per cent, "average" resolution in 45 per cent, and "bad" resolution in 18 per cent of the chromatograms.

The nine peaks were analyzed for their steroid components by three methods: 1) comparison with elution behavior of pure compounds on silica gel, 2) infrared analysis of pooled materials from the peaks obtained from normal urine, and 3) comparison with the paper chromatographic behavior of pure compounds. The infrared absorption curves of the urinary fractions were compared with the spectra of standard alpha-ketosteroids. In 2 instances, positive identification of steroid compounds in the urinary extracts was possible. When specific identity was not established, certain steroid metabolites could be eliminated as possibilities by the infrared data. The paper chromatographic studies not only added evidence for identity of some of the compounds, but gave further resolution of some of the peaks into two or more components. In Table 3 are listed the components of the nine silica-gel fractions, according to all the available evidence.

Peak I. Peak I was eluted by mixtures which would elute $\Delta^{2 \text{ or } 3}$-androstene-17-one and 3β-chloro-Δ^5-androstene-17-one.

The infrared spectrum contained a strong band near 5.75 μ, indicative of a C-17 carbonyl group. No hydroxyl group absorption was present. A contaminant gave rise to a weak carbonyl band near 5.85 μ. No distinct bands appeared between 9 and 11 μ because background material obliterated them. Some similarity was seen upon superposition of the spectrum of $\Delta^{2 \text{ or } 3}$-androstene-17-one, but identification was not complete.

Paper chromatography, using a heptane-phenyl cellosolve system, resolved peak I into two spots, one moving at the same rate as $\Delta^{2 \text{ or } 3}$-androstene-17-one and one at the same rate as 3β-chloro-Δ^5-androstene-17-one.

Peak II. A number of absorption bands were evident between 7.5 and 9 μ in the infrared spectrum. Again, a C-17 carbonyl was present and hydroxyl absorption was lacking. The spectrum differed from that of Peak I. It was also not identical with the spectra of *i*-androstane-6-ol-17-one, 3β-chloro-Δ^5-androstene-17-one, dehydroepiandrosterone, Δ^4-androstene-3,17-dione, or $\Delta^{3,5}$-androstadiene-17-one.

The material in Peak II moved on paper at a rate slower than those of $\Delta^{2 \text{ or } 3}$-androstene-17-one and 3β-chloro-Δ^5-androstene-17-one and faster than those of the other compounds listed. Actually, the presence of *i*-androstane-6-ol-17-one could not be expected, since it would be converted to dehydroepiandrosterone during the hydrolysis of the steroid conjugates with heat and strong acid.

Peak III. The small peak designated as Peak III on the graphs in Figure 3 is an artefact of elution. The infrared spectrum of Peak III was the same as that of Peak II. The residual steroid of Peak II, which had

been coming off the column at a slow rate in 1:49 ether-benzene, was eluted more rapidly when the eluent mixture was changed to 1:19 ether-benzene. Androsterone was present in low concentration in some of the pooled fractions studied in the infrared spectrometer. There was no evidence in the infrared spectrum for the presence of pregnane-3α-ol-20-one the only crystalline steroid studied which was eluted by the first 120 ml. of 1:19 ether-benzene.

Peak IV. Peak IV was eluted from the column at the same position as androsterone.

This fraction was clearly identified as androsterone by its infrared spectrum, which showed absorption bands near 9.5, 9.78, and 9.88 μ.

TABLE 3. ANALYSIS OF SILICA-GEL PEAKS OBTAINED FROM α-KETONIC EXTRACTS OF URINES FROM NORMAL MEN, AGED 20–36

Silica-gel peak No.	Known steroid eluted by same eluent	Analysis of silica-gel peaks		
		Infrared	Paper chromatography	
			Solvent system	Steroids indicated
I	$\Delta^{2 \text{ or } 3}$-androstene-17-one	Partly $\Delta^{2 \text{ or } 3}$-androstene-17-one	Heptane-phenylcellosolve	Two spots, one moving at same rate as $\Delta^{2 \text{ or } 3}$-androstenone and one at the same rate as 3β-chloro-Δ^5-androstene-17-one
II	Unknown	Different from I. Identity unknown	Heptane-phenylcellosolve	Material moving at a rate slower than those of $\Delta^{2 \text{ or } 3}$-androstene-17-one, and 3β-chloro-Δ^5-androstene-17-one; and faster than those of *i*-androstane-6-ol-17-one, Δ^4-androstene-3,17-dione, and $\Delta^{3,5}$-androstadiene-17-one
III	Pregnane-3α-ol-20-one	Same as II. Identity unknown	Heptane-propylene glycol	Material moving faster than pregnane-3α-ol-20-one
IV	Androsterone	Primary component, androsterone. Etiocholane-3α-ol-17-one contaminant	Heptane-propylene glycol	Major component moving at same rate as androsterone; minor component moving at the same rate as etiocholane-3α-ol-17-one
V	Etiocholane-3α-ol-17-one	Primary component, etiocholane-3α-ol-17-one	Heptane-propylene glycol	Major component moving at same rate as etiocholane-3α-ol-17-one; minor component moving at same rate as androsterone
VI		Etiocholane-3α-ol-17-one mixed with an unidentified component	Heptane-propylene glycol	Material moving at same rate as etiocholane-3α-ol-17-one
VII		Major component, the unidentified component of Peak VI	Heptane-propylene glycol	Component moving at rate slower than that of etiocholane-3α-ol-17-one and faster than that of 11-hydroxy-androsterone
VIII	1-Hydroxy-androsterone	Same as VII	Heptane-propylene glycol	Component moving at same rate as VII, plus a slower component
IX		Too impure for analysis	Heptane-propylene glycol	Component moving at same rate as slower component from VIII.

Approximately 75–85 per cent of the material was androsterone. Etiocholane-3α-ol-17-one was a minor constituent of this fraction.

Further evidence that Peak IV was contaminated with etiocholane-3α-ol-17-one was obtained from paper chromatography. Pooled fractions were chromatographed with both the ligroin-propylene glycol system and the heptane-propylene glycol system. Two components were resolved. The mobility of the major component agreed with that of androsterone and the mobility of the minor component agreed with that of etiocholane-3α-ol-17-one.

Peak V. Peak V was eluted from the silica-gel column in a manner similar to that of etiocholane-3α-ol-17-one.

It was possible by infrared analysis to identify positively the major component of Peak V. Between 75 and 85 per cent of the material was shown to be etiocholane-3α-ol-17-one. There were absorption bands near 9.3, 9.63, and 9.93 μ.

Paper chromatography, by the ligroin-propylene glycol and heptane-propylene glycol systems, showed that the pooled material had a major component moving at the same rate as etiocholane-3α-ol-17-one and a minor component moving at the same rate as androsterone.

Peak VI. The concentration of steroids in this eluate was so low that the masking effect of the background material made positive identification impossible. A C-17 carbonyl group (5.76 μ) was predominant. Another compound in low concentration, containing a carbonyl group absorbing at 5.86 μ was also in evidence. Etiocholane-3α-ol-17-one was found in some of the fractions.

Paper chromatography of Peak VI demonstrated a steroid moving at the same rate as etiocholane-3α-ol-17-one.

Because of the presence of etiocholane-3α-ol-17-one as a component of this peak, Peak VI was also considered to be in part an artefact of elution, and the 17-ketosteroid values for Peak VI were added to those of the etiocholane-3α-ol-17-one.

Peaks VII, VIII and IX. The only crystalline material studied on silica gel which was eluted in this region was 11β-hydroxyandrosterone.

The infrared spectra of Peaks VII and VIII were similar to that of Peak VI. A C-17 ketone was present and there was also another carbonyl-containing minor component. Peak IX had too much nonsteroid background material for valid steroid analysis.

Peak VII contained a component moving on paper at a rate slower than those of etiocholane-3α-ol-17-one or of Δ^4-androstene-3,11,17-trione (adrenosterone), and faster than that of 11-hydroxyandrosterone. Peak VIII yielded this same component, plus another component, moving more slowly than that of Peak VII, and Peak IX yielded only this last com-

ponent. Both of these components moved at rates faster than those of either 11β-hydroxyandrosterone or etiocholane-3α,11β-diol-17-one. There were no compounds corresponding to either 11β-hydroxyandrosterone or or etiocholane-3α,11β-diol-17-one. Some Zimmermann-reacting material could still be detected at the starting line of the paper strip after ninety-six hours.

Peaks VIII and IX were also considered to be artefacts of elution, arising from a shift to more polar eluent mixtures.

DISCUSSION

The percentile distribution of the various peaks, or pattern of steroid excretion, is tabulated in Table 4. The artefact materials (Peak I) made up 4.6 ± 0.6 per cent of the total. Peaks II and III represented 12.5 ± 1.3 per cent. There was a smaller amount of androsterone (Peak IV) (24.6 ± 1.0 per cent) than of etiocholane-3α-ol-17-one (Peaks V and VI) (35.8 ± 2.8 per cent). This difference is statistically significant (Fisher's t value of 3.29) (15). The compounds more polar than etiocholane-3α-ol-17-one (Peaks VII, VIII and IX) accounted for 18.7 ± 1.1 per cent of the total 17-ketosteroids.

The patterns of distribution of 17-ketosteroids in normal male urine as determined by aluminum-oxide chromatography in other laboratories are also tabulated in Table 4. The data of Dingemanse *et al.* (16) and of Robinson and Goulden (6) were recalculated in terms of the percentage of the total alpha-ketosteroids represented by each of their components, and the standard errors and significance of the differences between their mean values and those obtained using silica gel were also calculated. For urines from men aged 17–41 years, several significant differences in pattern appear when the different methods of chromatography are used. Both Dingemanse *et al.* and Robinson and Goulden found a higher percentage of artefact compounds ($\Delta^{2 \text{ or } 3}$-androstene-17-one and 3β-chloro-Δ^5-androstene-17-one) than was found in this study. Because of the hydrolytic procedure (heat and strong acid) which was used in this laboratory, *i*-androstane-6-ol-17-one was not expected to be present in the alpha-ketonic fractions. Dingemanse and associates, who extracted urine at neutral pH, reported 12.5 per cent of the alpha-ketosteroids to consist of this compound. The amount of material in our unidentified Peak II is 12.5 ± 1.3 per cent. Dingemanse *et al.* make no mention of finding another component in their chromatograms eluted in this same region. They, as well as Robinson and Goulden found more material in the androsterone peak than was found here, but the Dingemanse group found significantly less material in the etiocholane-3α-ol-17-one peak than we did.

TABLE 4. PATTERN OF DISTRIBUTION OF 17-KS IN NORMAL MEN

Reference	Age range (yrs.)	Duration of urine collection (hrs.)	No. of subjects	Steroids—in order of elution													
				Hydrolysis artefacts: Δ^{2} or $_{1}$-androstene-17-one; 3β-chloro-Δ^{5}-androstene-17-one		t-Androstane-6-ol-17-one	Unidentified	Androsterone; Δ^{4}-androstene-3α-ol-17-one		Etiocholane-3α-ol-17-one; Δ^{4}-etiocholene-3α-ol-17-one		11β-hydroxyandrosterone	Etiocholane-3α,11β-diol-17-one	Others	Sum of substances more polar than etiocholane-3α-ol-17-one	
				Total α* ± S.E. %	t**	Total α* ± S.E. %	Total α* ± S.E. %	Total α* ± S.E. %	t**	Total α* ± S.E. %	t**	Total α* ± S.E. %	Total α* ± S.E. %	Total α* ± S.E. %	Total α* ± S.E. %	t**
This paper	20–36	24	12	4.6±0.6			12.5±1.3	24.6±1.9		35.8±2.8				18.7±1.1	18.7±1.1	
Dingemanse *et al.* (16)	18–41	*ca.* 4.8†	29	10.6±0.7	4.35	12.3±1.7		33.7±1.4	3.85	22.1±1.2	4.50	15.3±0.9		7.2±0.9	22.5±1.5	2.03
Robinson and Goulden (6)	17–37	24	7	15.6±2.1	5.21	2.0±0.9	1.0±0.5	35.6±3.6	2.69	28.4±2.9	1.83	5.6±1.2	8.2±0.8	3.3±0.6	17.1±1.2	0.98
	11–73	24	18	14.7±1.3		2.7±0.6	0.8±0.3	33.2±2.6		27.3±3.2		5.3±1.3	11.4±1.8	3.7±0.4	20.4±2.5	
Devis (7)	18–45	72	40	3–30‡		0–21‡				32–60‡		3–23‡		2–20‡		

* Calculated after subtracting β-KS values from data of Dingemanse *et al.* (16) and of Robinson and Goulden (6).

** Significance of difference between reference's mean and author's mean.

† Aliquot of 72-hour collection.

‡ Values are the range (in per cent) of total 17-KS. No data available for calculating per cent (α) or S.E.

Unlike Dingemanse *et al.* (16), Robinson and Goulden (6), Pond (9) and Devis (7), we did not find any measurable amount of 11β-hydroxyandrosterone or etiocholane-3α,11β-diol-17-one. It is possible that these compounds were dehydrated by our hydrolytic procedure, involving strong acid and boiling, and that the resultant unsaturated compounds were then eluted along with the androsterone and etiocholane-3α-ol-17-one. Preliminary studies indicate that the paper chromatographic technique, which separates androsterone most effectively from etiocholane-3α-ol-17-one, will not resolve a mixture of either of these with its Δ-9 analogue. It was not possible to determine the presence of unsaturated compounds in the androsterone and etiocholane-3α-ol-17-one peaks by infrared analysis, because of their low concentration. The only comparison which can be made of the compounds more polar than etiocholane-3α-ol-17-one is to compare the sum of the 11-hydroxy compounds reported by Dingemanse and co-workers (16) and by Robinson (6) with the sum of the more polar compounds (sum of Peaks VII, VIII and IX) obtained from silica gel. On this basis, no significant differences are observed. A true measure of the more polar compounds is difficult, because of the unknown amounts of Δ-[9] compounds arising from them and being eluted in the androsterone and etiocholane-3α-ol-17-one fractions.

SUMMARY

A routine procedure has been devised for separating the neutral ketonic, digitonin-nonprecipitable steroids in a 24-hour urine sample by silica-gel chromatography. Nine peaks were obtained, three of which were artefacts of elution. Identification studies were made on the components of the peaks by comparing them with the behavior of crystalline compounds in the standard chromatographic procedure and in paper chromatography, and by infrared analysis. The urines of 12 normal young men (age 20–36) were studied. A comparison is made of the pattern of excretion in normal men as determined by silica-gel chromatography and that obtained by other workers utilizing aluminum oxide.

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AN IMPROVED METHOD FOR DETERMINATION OF URINARY 17-KETOSTEROIDS*

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INTRODUCTION

IN THE determination of urinary 17-ketosteroids, it has been recognized that interfering chromogens may cause significant errors. Three methods have been introduced to eliminate or reduce these errors. Talbot *et al.* (1) have shown that these chromogenic materials are largely in the nonketonic fraction of crude urinary extract. The use of Girard's reagent T results in a chemical separation which eliminates this fraction, but it is a time-consuming procedure.

The use of a correction equation in methods involving aqueous alkali has been shown by Engstrom and Mason (2) to introduce some serious errors. Since there appears to be no constant relationship between the amounts of 17-ketosteroids and of nonketonic chromogenic substances, there may also be qualitative differences which may not be evident in the absorption maxima at the wavelengths used in various correction equations, indicating that such a rigid formula may not be applicable in every case.

Cahen and Salter (3) used a relatively simple method of extracting the final pink Zimmermann color with chloroform, the undesirable pigments then remaining in the aqueous layer. The chloroform was objectionable because of the turbidity encountered. Zimmermann *et al.* (4) have recently reported a method utilizing ether or chloroform extraction of the Zimmermann color. Using identical samples, their extraction values were in close agreement with those obtained on the ketonic fraction separated with the aid of Girard's reagent. Their results also emphasized the unreliability of the various color correction formulae.

This paper describes a method for estimation of total neutral 17-ketosteroids, which utilizes amyl acetate as a solvent for the final Zimmermann color.

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METHOD

Hydrolysis, extraction and Zimmermann reaction are carried out by a modification of the method of Drekter *et al.* (5). This method, which is rapid, reproducible and requires only small amounts of urine for analysis, is as follows: To a 10-cc. sample of urine, is added 3 cc. of concentrated HCl. The temperature is brought up to 80° C. in a water bath, and kept at 80–85° C. for ten minutes. The hydrolyzed urine is cooled rapidly, and 10 cc. of the mixture is extracted with 40 cc. of absolute ether, shaking for thirty seconds. The ether extract is washed once with 20 cc. of 10 per cent NaOH and once with 20 cc. of distilled water, shaking for ten seconds. Six 5-cc. aliquots of the ether extract are pipetted into test tubes and evaporated to dryness in a water bath.

To each dried extract is added 0.2 cc. of absolute alcohol, 0.3 cc. of aqueous 5N KOH, and 0.2 cc. of 2 per cent *m*-dinitrobenzene in absolute alcohol. The mixture is shaken and placed in a constant temperature bath in the dark at 27° C. for ninety minutes. At the end of this time, the tube is removed and 1 cc. of 75 per cent alcohol is added and shaken; then 1 cc. of amyl acetate is added. The tube is shaken, twirled and shaken vigorously for ten seconds and then placed in the dark for eight minutes. At this time, the tube is removed from the dark. A pipette fitted with a rubber bulb is used to transfer the supernatant amyl acetate-alcohol layer into a cuvette. The optical density is read immediately in a Beckman spectrophotometer at 510 mμ. The final color is in the organic solvent; the remaining aqueous layer contains undesirable pigments (a deep yellow or brown). The colorimetric determination as outlined here is carried out on three sample aliquots. To each of the remaining three aliquots is added 0.2 cc. of the standard solution instead of the absolute alcohol. The difference between the readings on these two types of aliquot is taken as the value for the standard. The standard used was dehydroisoandrosterone in absolute alcohol, of such concentration that 0.2 cc. was equivalent to 20 micrograms.

In actual practice, when 45 determinations or less are made, the tubes are staggered at two-minute intervals, with the reaction timing begun in each case by the addition of the *m*-dinitrobenzene. When making many determinations, it is practical to premix the needed amounts of 5N KOH and absolute alcohol, or 5N KOH and standard solutions, and to pipette 0.5 cc. of this fresh mixture instead of 0.3 cc. and 0.2 cc., respectively.

EXPERIMENTAL

Other organic solvents tried were benzene, ether, carbon tetrachloride and trichloroethylene. These were rejected for various reasons, such as poor extracting power, turbidity and volatility. Normal amyl acetate was chosen, since it appeared to extract all the color, gave no turbidity, and the separation of the organic from the aqueous layer was rapid.

Total 17-ketosteroid determinations were made on 14 different urine samples, using both the Drekter method and the amyl acetate (AA) method. Table 1A shows that there is a definite reduction in titer, using the latter method. This reduction, which ranges from 5.7 to 37.9 per cent, is comparable to that found by Cahen and Salter (3) with chloroform extraction, and by Pincus (6, 7) with both his antimony trichloride reagent, and micro-Girard procedures (Table 1B).

Wilkins and Carlson (8) found in chromatographic fractionation of 17-ketosteroids that the sum of the fractions was less than that obtained on

routine analysis by the Drekter method, and that this difference was greatest when diuresis was effected by forced fluids. This reduction seems to be due to the adsorption of interfering pigments and chromogens by the alumina used in the column. Elimination of these pigments and chromogens would give a more accurate value for the total 17-ketosteroids. Figure 1A shows the values for 17-ketosteroids in extracts from 3 normal urines; each extract was analyzed by the Drekter method, by the AA method and by chromatography.

TABLE 1

A. COMPARISON OF 17-KS TITERS OF URINE EXTRACTS, DETERMINED BY DREKTER AND BY AA METHODS.

All totals are averages of 3 determinations.

Sample	Drekter (mg./hr.)	AA (mg./hr.)	$\frac{AA}{D} \times 100$ (%)
C1	1.131	.875	77.4
C4	.988	.792	80.2
C5	.889	.719	80.9
C6	.841	.656	78.0
M	1.181	.787	66.6
C	2.070	1.695	81.9
L	.038	.029	76.3
U	2.436	1.938	79.6
J	2.737	2.580	94.3
Ma	1.213	1.141	94.1
M1	1.425	1.125	79.0
M2	1.228	.910	74.1
M3	2.281	1.720	75.4
M4	2.088	1.296	62.1

B. COMPARISON OF AA TITERS WITH THOSE BY OTHER METHODS OF 17-KS ESTIMATION, EXPRESSED AS PERCENTAGE OF TITER BY ORIGINAL ZIMMERMANN METHOD.

AA	Cahen and Salter	Pincus, $SbCl_3$	Pincus, micro-Girard
62.1–94.3	68.6–75.0	60.0–68.0	43.0–93.0

The use of Girard's reagent is a recognized procedure which separates the ketonic from the nonketonic material in crude urine extracts. Although small amounts of material may be lost by partial hydrolysis and manipulation, Pincus and Pearlman (7) report good recoveries in their experi-

ments, and this chemical purification of the extract gives a more accurate estimation of the urinary 17-ketosteroids. Figure 1B shows the total 17-ketosteroids in 4 urine extracts, as estimated by the Drekter method, by the AA method, and after Girard separation.

To determine whether the final Zimmermann color in the AA method follows Beer's law, different concentrations of dehydroisoandrosterone,

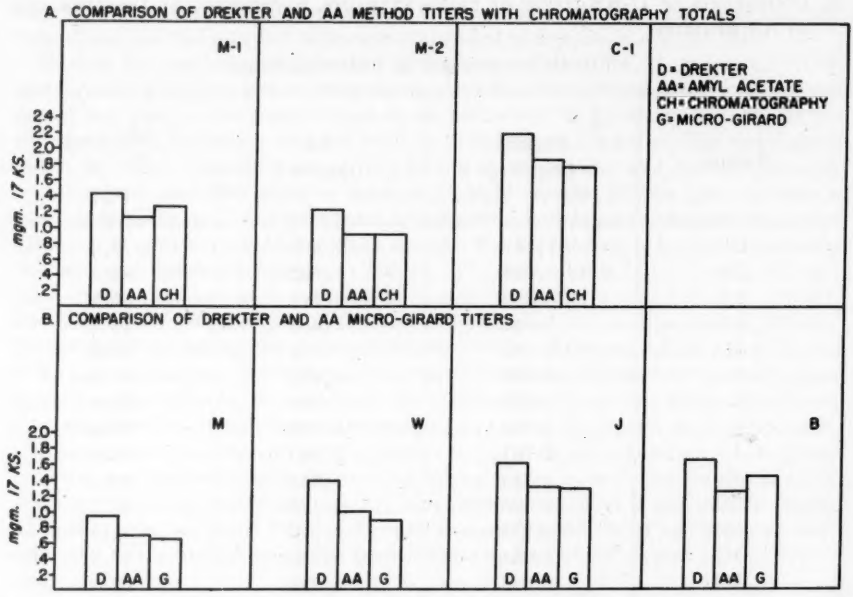

FIG. 1A. Total 17-ketosteroids of 3 normal urine samples, each analyzed by the Drekter method, by the AA method, and by chromatography.

FIG. 1B. Total 17-ketosteroids of 4 urine extracts, each estimated by the Drekter method, by the AA method, and after Girard separation.

androsterone, androstanedione-3,17, and urine extract were analyzed. Figure 2 shows the results obtained by plotting optical density against concentration of 17-ketosteroids and volume of urine extract.

Sloan and Lowrey (9) demonstrated in the urine extract some substance which inhibits the color produced in the Zimmermann reaction. Recovery experiments, using the AA method, indicate that the greater the optical density of the urine extract, the less the recovery of added 17-ketosteroid or the greater the inhibition of color. Therefore, the suggestion of Sloan and Lowrey of using as the reference the difference in the reading between the standard and the standard plus extract, has been followed throughout.

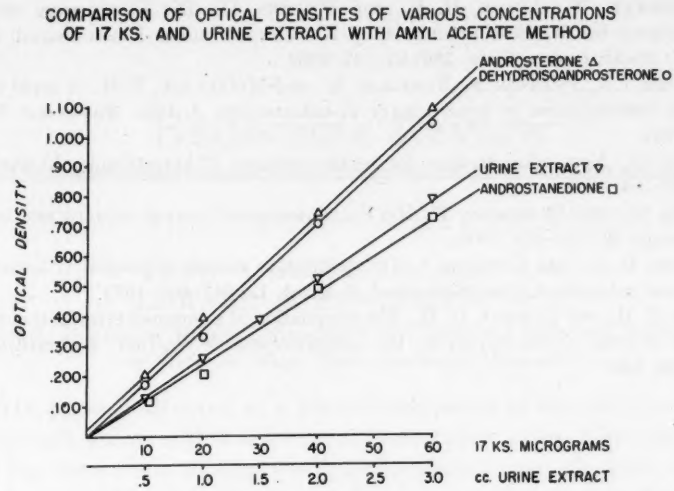

FIG. 2. Results obtained plotting optical density against concentration of 17-ketosteroids and volume of urine extract.

SUMMARY

1. A simple method of estimation of total neutral 17-ketosteroids is given, in which the final Zimmermann color is extracted with amyl acetate. The extraction with organic solvent leaves behind in the aqueous layer the nonketonic interfering chromogens.

2. The definite reduction in titer by this method is comparable to the reduction in titer after micro-Girard separation and chromatography.

3. Pure 17-ketosteroids and urine extracts assayed by this method show linearity according to Beer's law.

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Endocrine Review

MEDICAL ASPECTS OF HYPOGLYCEMIA

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THIS paper is devoted to a general discussion of the various types of hypoglycemia, with a report of representative cases. Elsewhere (1) an effort has been made to separate hyperinsulinism from all other causes of hypoglycemia. The term "hyperinsulinism" should be reserved for those patients in whom a hyperfunctioning tumor of the islets of Langerhans has been demonstrated, or who have received an injection of an excessive amount of insulin. Many other causes of hypoglycemia will be found in the classification by Conn (2) (Table 1), some of which are discussed later in the paper. The term "functional hypoglycemia" as defined by him (2) refers to a disturbance in the nervous regulation of the concentration of blood sugar, manifested by hypoglycemic symptoms following meals of average or high carbohydrate content in a patient with increased emotional instability. Throughout this paper, the values given for blood sugar were determined by the Folin-Wu method.

As Wilder (3) has pointed out, the clinical symptoms of the hypoglycemic state are constant, regardless of the cause. These symptoms are mostly of a psychic or neurologic character, owing to the effects of hypoglycemia on the central nervous system. Various authors (4–7) have classified the symptoms of hypoglycemia into various stages, according to the order of their development. Moersch and Kernohan (6) described three stages of symptoms. The first, that of retardation, may last ten to thirty minutes, during which the patient seems mentally and physically retarded. There is increasing lassitude with beginning pallor and mild mental confusion. During this period irritability, restlessness, transitory diplopia and increasing confusion may be present. The blood pressure, pulse and respirations may be unaltered. Drowsiness may be the outstanding symptom.

The second stage is described as one of excitement and is manifested by

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TABLE 1. ETIOLOGIC CLASSIFICATION OF SPONTANEOUS HYPOGLYCEMIA*

I. Organic—recognizable anatomic lesion
  A. Hyperinsulinism
    1. Pancreatic island cell adenoma
      (a) Single
      (b) Multiple
      (c) Aberrant
    2. Pancreatic island cell carcinoma
      (a) Localized
      (b) With metastases
    3. Generalized hypertrophy and hyperplasia of the islands of Langerhans
  B. Hepatic disease
    1. Ascending infectious cholangiolitis
    2. Toxic hepatitis
    3. Diffuse carcinomatosis
    4. Fatty degeneration or "fatty metamorphosis"
    5. Glycogenosis (von Gierke's disease)
  C. Pituitary hypofunction (anterior lobe)
    1. Destructive lesions (chromophobe tumors, cysts)
    2. Atrophy and degeneration (Simmonds' disease)
    3. Thyroid hypofunction (? secondary to pituitary hypofunction)
  D. Adrenal hypofunction (cortex)
    1. Idiopathic cortical atrophy
    2. Destructive infectious granulomas
    3. Destructive neoplasms
  E. Central nervous system lesions (hypothalamus or brain stem; interference with nervous control of blood sugar)
II. Functional—no recognized anatomic lesion but explainable on basis of unusual somatic function
  A. Hyperinsulinism (imbalance of the autonomic nervous system): hypoglycemic fatigue; nervous hypoglycemia; functional hypoglycemia; reactive hypoglycemia
  B. Alimentary hyperinsulinism (rapid intestinal absorption)
    1. After gastroenterostomy
    2. After gastric resection (partial or total)
  C. Renal glycosuria (severe degrees of low renal threshold for dextrose)
  D. Lactation
  E. Severe continuous muscular work
III. Miscellaneous
  A. Factitious (surreptitious insulin administration)
  B. Postoperative hypoglycemia
  C. Severe inanition
  D. Unknown

* Reprinted with permission from Conn, J. W.: Diagnosis and management of spontaneous hypoglycemia, *J.A.M.A.*, **134:** 130–138 (May 10) 1947. (2)

stimulation and confusion, with marked pallor, profuse perspiration, restlessness and increasing confusion. The agitation may increase to delirium or mania, and all types of bizarre psychic behavior have been observed.

The third stage is that of coma, characterized by complete stupor with or without convulsions. During this stage patients may have the signs and symptoms typical of a disorder of the central nervous system, including positive Babinski reflexes, nystagmoid movements of the eyes, motor palsy, hemiparesis, and so forth. These symptoms usually disappear after recovery from the hypoglycemia.

The physiologic mechanism by which hypoglycemia produces these symptoms is not well understood, but most investigators (6, 8–10) have expressed the belief that it is based on a depression of the oxidative processes in the brain, so that the cells are affected in the same manner as by anoxia due to other causes. Evidence of this effect of hypoglycemia upon the brain cells can be recorded by the electroencephalograph. The electroencephalographic changes are of a nonspecific nature similar to those seen in many other disorders producing a state of anoxia in the cells of the central nervous system. Fabrykant and co-workers (11–13) have published several papers recently which delve into the effects of hypoglycemia on the central nervous system, in more detail than can be presented here.

Certain pathologic changes have been noted in postmortem examinations that followed severe insulin reactions. These changes are strikingly similar to those seen in the brain of patients who die of cerebral anoxia. Hoff and co-workers (9), Wolf (10), and Moersch and Kernohan (6) have said that the most important changes that occur are degeneration of the nerve cells and multiple petechial hemorrhages. Wolf (10) has pointed out that early degenerative changes in the nerve cells such as chromatolysis, as well as the symptoms of hypoglycemia, are reversible if glucose is given early enough. If the hypoglycemic state persists long enough, certain irreversible degenerative changes may occur, resulting in necrosis and destruction of nerve cells and lack of relief of the symptoms following administration of glucose by vein (14). One of our diabetic patients, a 15-year-old boy who had been given too much insulin, upon admission demonstrated complete decerebrate rigidity from which he did not recover. At necropsy, examination of the brain disclosed numerous petechial hemorrhages throughout the cortex and one large hemorrhage. Some of our patients with islet cell tumors have demonstrated chronic mental deterioration but in most instances marked improvement in regard to these mental changes followed removal of the tumor.

HYPOGLYCEMIA DUE TO EXCESS INSULIN

One of the most important causes of hypoglycemia is the presence of an excess of insulin. This may originate from an endogenous source by secretion from a functioning islet cell neoplasm of the pancreas, or from an exogenous source by injection.

Therapeutic use of insulin.—The most common exogenous type of hypoglycemia is seen in the diabetic patient who is having a reaction following an overdose of insulin. Hypoglycemia is also produced by large doses of insulin given as a therapeutic measure in the treatment of certain psychotic patients.

Surreptitious injection of insulin.—The patients who are the most difficult to distinguish from those with true hyperinsulinism due to islet cell tumor of the pancreas, are those who have produced hypoglycemia by the surreptitious injection of insulin. Conn and co-workers (15) first described this condition in 1946. We have encountered 6 patients of this type in the past eight years. Three of these patients have already been reported (16).

The common denominator of these 6 patients is that each had had previous experience with insulin—3 as diabetic patients and 3 as nurses. Probably another common denominator could be designated "a disturbed psychic state," for certainly it is not part of a normal behavior pattern to inject so much insulin into one's self that severe insulin reactions are produced. One of these patients had submitted to a total of seven exploratory operations, in search for a functioning adenoma, before she was found to be surreptitiously using insulin. She was the first patient to "fool" us in this fashion—the result of a combination of years of study and stealth on her part, and a "low index of suspicion" on ours. She and 2 other patients had become familiar with the use of insulin in the treatment of their diabetes and then "fooled" their families and physicians by declaring that they continued to have severe reactions long after they stopped taking insulin.

These 3 patients and the 3 nurses all were exceedingly adept in hiding their supplies of insulin. One used the toilet reservoir, another suspended the equipment on a string outside the window, another used the space in the bottom of a vacuum-bottle container. Even when confronted with the evidence, patients of this type may persistently deny having misused insulin. Conn and co-workers (15) proved self-administration by adding typhoid vaccine to the patient's bottle of insulin, thus producing a different kind of reaction! Keating (16) added a radioactive solution to the patient's insulin and proved self-administration by measuring the radioactivity of the urine. It can be seen that if the diagnosis is to be made, the physician must also be a detective! When the diagnosis is made it is important to seek psychiatric consultation immediately, rather than to confront the patient directly with the evidence.

Keating and Wilder (17) have reported 2 instances in which hypoglycemia developed in diabetic patients as the result of severe disease of the liver. Lopez-Kruger and Dockerty (18) reported the finding of three nonfunctioning islet cell adenomas in diabetic patients but we have seen no instance in which hyperinsulinism developed in a diabetic patient. We have had only 1 nurse who has had a tumor of the islet cells. Consequently we shall continue to "view with suspicion" any of our patients who by personal experience or professional training have had the opportunity to learn the drama of a severe insulin reaction.

Functioning tumor of the pancreas.—The most important group of patients with hypoglycemia are those having hyperinsulinism due to a functioning tumor of the pancreas. Harris (19) in 1924 first suggested the possibility that hyperinsulinism could be due to hypersecretion of insulin by islet cell tissue. Wilder and his associates (20) in 1927 reported the first case in which this clinical syndrome was recognized and in which an excess of insulin was found in the tumor tissue. Since then many excellent papers have appeared in the literature, including those of Whipple and Frantz (21), Clyne and co-workers (22), Hanno and Banks (23), Frantz (24), Holman and co-workers (25), David and Campbell (26), Keating and Wilder (17), and Lopez-Kruger and Dockerty (18). Crain and Thorn (27) have presented an extensive statistical analysis of data on 259 cases reviewed through January 1, 1949. Howard and co-workers (28) have recently reviewed the data on 398 cases of islet cell tumors of the pancreas.

In this discussion we shall not repeat the statistical summaries from these reviews, nor shall we tabulate our experience with 65 patients seen from 1927 to 1951 who were found to have hyperinsulinism due to secreting adenomas of the pancreas. Lopez-Kruger and Dockerty (18) have reviewed the pathologic findings in the first 38 of these patients.

We should like to emphasize the importance of Whipple's triad (29) in the diagnosis of these tumors. First, the patient gives the story that his attacks are clearly associated with fasting or with exercise, or with both. Second, during an attack the concentration of blood sugar is usually less than 50 mg. per 100 cc. Third, the attacks are promptly relieved by the administration of glucose by mouth or by vein.

The most helpful test in confirming the diagnosis is to withhold food and have the patient exercise. We prefer to have this conducted in the hospital where close supervision is possible, where the blood specimens can be drawn with ease and where treatment can be instituted promptly. Almost all patients with functioning tumors will have their attacks within a few hours of the withholding of food; in some cases, however, more than forty-eight hours must elapse before the attacks occur, and we sometimes continue the fast for seventy-two hours if attacks fail to occur in the mean-

time. We have records of having conducted a fast in 47 of our 65 patients, and only 1 patient was able to go without food for more than forty-eight hours without the development of hypoglycemia; on a second test she had a typical attack in forty-three hours.

In our experience, the sugar tolerance test has been of no value. It is true that almost all of these tests were performed with the oral administration of glucose but we have seen no evidence to indicate that any modification of the sugar tolerance test makes it as helpful as a fast. Crain and Thorn (27) state: "Probably the most confusing finding in the group of patients with insulinomas is the character of the glucose tolerance test. No two investigators have conducted the tests similarly. Results vary from flat curves to diabetic types. . . . " Nor has the insulin tolerance test proved to be of any help; too few have been reported for statistical analysis.

We have already referred to the clinical symptoms of hypoglycemia. We concur with Kepler and Moersch (30) who commented upon the ease of diagnosing hyperinsulinism in reviewing the histories of proved cases, as contrasted with the difficulty of diagnosing it when the patient is first seen. Almost all of these patients have had their symptoms for years; the diagnosis is not made because it is not suspected. Once the nature of the disease is suspected, confirmation is usually very easy. The symptoms are so transitory and bizarre that even the patient may not be aware of them. Sweating is more often an objective finding than a subjective symptom and may not be mentioned voluntarily by the patient. Twenty-five per cent of the patients whose records were reviewed by Crain and Thorn had had symptoms for more than five years. When the tumor was malignant the symptoms were usually more severe and more rapidly progressive and the diagnosis made much earlier than when the tumor was not malignant.

We should like to emphasize again the preponderance of neurologic and psychic symptoms which these patients experience. One of our most recent patients, a girl 14 years of age, experienced every morning a right hemiplegia which interfered with dressing and which was promptly relieved by breakfast. Many patients have had severe and serious psychotic disturbances. Table 2 lists the signs and symptoms of 193 patients with insulinomas; virtually every symptom is related to involvement of the brain.

Once the diagnosis of hyperinsulinism has been made with as much certainty as possible, the patient should be urged to have an operation for removal of the tumor or resection of the pancreas. This presupposes that the other causes of hypoglycemia, to be discussed later, have been eliminated; for if the story of hyperinsulinism is unquestionable, then one or more hyperfunctioning tumors must be present.

TABLE 2. SIGNS AND SYMPTOMS EXHIBITED BY 193 PATIENTS WITH INSULINOMAS*

Sign or symptom	Per cent
Loss of consciousness	58
Confusional state	54
Weakness and fatigue	41
Deep coma	40
Sweating	36
Drowsiness and stupor	35
Lightheadedness	30
Visual disturbances	30
Amnesia	28
Clonic convulsions	24
Noisy behavior	20
Headache	20
Tremor	18
Hunger	14
Positive Babinski sign	13
Paresthesias	13
Irritability	11
Transient hemiplegia	10
Abdominal pain	8
Palpitation	3

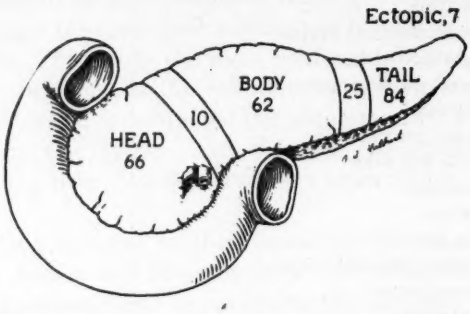
* Reprinted with permission from Crain, E. L., Jr. and Thorn, G. W.: Functioning pancreatic islet cell adenomas; a review of the literature and presentation of two new differential tests, *Medicine* **28**: 427–447 (Dec.) 1949. (27)

There is nothing more gratifying than to find at operation an easily removable insulinoma. The following case is typical of hyperinsulinism due to a single adenoma of the pancreas.

A 41-year-old man had first noticed blurring of vision at the end of a golf game five years previously; this was relieved by food. Two years previously he first experienced episodes of coma that occurred before meals. He was also confused and dysarthric on arising early in the morning. During milder episodes he would become very stubborn. He had an automobile accident without any recollection of what had happened. Food always relieved his symptoms. At the time of examination the value for fasting blood sugar in the morning was 45 mg. per 100 cc. During two attacks the concentration of blood sugar was 26 and 38 mg. respectively. A fast was terminated in six hours owing to marked mental confusion; the value for blood sugar at that time was 45 mg. At operation an adenoma was removed from the tail of the pancreas. Three daily postoperative determinations of fasting blood sugar were 190, 185, and 125 mg., respectively, reaching normal values one week later. No further symptoms were experienced.

Often, however, the tumor cannot be identified by even the most careful search. It is then that the surgeon must proceed with radical resection. Figure 1 from Howard and co-workers (28) shows the location of these

tumors and illustrates why the surgeon should remove as much of the pancreas as possible if he is to have the best chance of removing "hidden adenomas." One patient first operated upon in 1928 (31) had three other resections performed by Drs. Judd and Walters, all without benefit. The patient continued to have severe symptoms and finally died in 1948. At necropsy a small benign adenoma was found in the small remnant of pan-



Overlooked at operation	Head	Body	Tail	Ectopic
	8	3	6	3

FIG. 1. Distribution of insulinomas. (Reproduced with permission of Surgery, Gynecology and Obstetrics from: Howard, J. M., Moss, N. H. and Rhoads, J. E.: Hyperinsulinism and islet cell tumors of the pancreas; with 398 recorded tumors, *Internat. Abstr. Surg.* **90**: 417–455 (May) 1950 (28).

creatic tissue. Because of this possibility we would urge total pancreatectomy in such an instance. Such a procedure was performed on 1 patient, who was found to have a small adenoma in the head of the pancreas (32). The patient is still living ten years after the operation and requires only a moderate amount of insulin. Another reason for "radical" resection is the incidence of multiple adenomas. Howard and co-workers (28) reported on 297 patients with single tumors and 43 with multiple tumors.

Underdahl, Woolner and Black (33) have reviewed the reports in the literature of 14 cases in which pituitary tumor, islet cell adenomas and parathyroid tumors, or combinations of two of these, have occurred in the same patient. They report 8 additional cases of this syndrome from the Mayo Clinic. Parathyroid tumors were present in all and pituitary tumors were present in 4 of their 8 cases. Five patients had islet cell tumors and the remaining 3 had hypoglycemia.

We have not encountered an instance in which hyperinsulinism was caused by diffuse hyperplasia. Such cases are included in reviews of this subject. A detailed study of these patients would suggest that in some in-

stances they did not have true hyperinsulinism in the first place, and that in others a small tumor might have been removed and not found at routine examination. In any event, in our series whenever a patient has been relieved of hyperinsulinism by resection of the pancreas we have been able to find the tumor. One of our patients was carried in our records for some years as having been relieved by subtotal pancreatectomy without a tumor having been found. At the end of this time Dr. R. G. Sprague, still suspicious, asked Dr. M. B. Dockerty to re-examine the removed tissue. He did so and found an adenoma that was only 2 mm. in diameter. In the last 27 adult patients to undergo exploration at the Mayo Clinic there were only 2 in whom a tumor was not found in the excised pancreatic tissue and in whom the symptoms were not relieved. We believe that these 2 patients will require further resection, for almost certainly they have hyperfunctioning adenomas.

A further reason for urging surgical excision is that this is the only known way by which we can save the lives of patients in whom the adenoma is already malignant, or in whom it may become so.

HEPATOGENIC HYPOGLYCEMIA

Another of the more important causes of organic spontaneous hypoglycemia is severe damage to the liver with failure of glycogenolysis. Mann and Magath (34) demonstrated the striking effect of hepatectomy on the concentration of blood sugar and the resultant hypoglycemic coma and death. It is our experience that the occurrence of hypoglycemia secondary to disease of the liver is extremely rare. When it has occurred, the hepatic damage has been severe. If there are hypoglycemic symptoms, they are usually secondary in importance to the symptoms of the hepatic disease and are usually terminal. Occasionally, however, the symptoms of hypoglycemia due to disease of the liver may be the primary complaint and must be considered in the differential diagnosis of the causes of hypoglycemia. One of the causes of hepatogenic hypoglycemia is malignant disease of the liver, either primary or metastatic. A number of case reports have been published (35–37). This cause of hypoglycemia should be differentiated, if possible, from that caused by islet cell carcinoma which has extensively involved the liver.

Other cases of hepatogenic hypoglycemia have been reported. Judd, Kepler and Rynearson (38) reported a case in which the condition was associated with fibrotic cirrhosis of the liver. It is interesting to note that the patient had been a diabetic for two years prior to the onset of hypoglycemic symptoms. Coller and Jackson (39) reported 3 cases in which the hypoglycemia was due to diffuse cholangitis secondary to disease of the biliary tract. Judd, Kepler and Rynearson (38) reported 1 case of hypoglycemia

due to fatty infiltration of the liver. We have observed hypoglycemia in cases of terminal infectious hepatitis. Stander (40) reported a case of toxic hepatic damage due to chloroform, and Cross and Blackford (41) reported a case of such damage due to neoarsphenamine, in both of which a pronounced hypoglycemic state occurred.

Hypoglycemia may be the presenting symptom in children with glycogenosis or von Gierke's disease. These patients usually have prominence of the abdomen due to enlargement of the liver. Mason (42) pointed out that the defect in carbohydrate metabolism is the inability of the liver, owing to its deficiency of alkaline phosphatase, to phosphorylate glucose to glucose 6-phosphate for utilization in the body. Keating and Wilder (17) reported a case of glycogenosis in a 17-year-old girl who had had diabetes mellitus for seven years prior to the onset of severe hypoglycemic symptoms.

HYPOGLYCEMIA DUE TO ANTERIOR PITUITARY OR ADRENAL INSUFFICIENCY

We are all aware of the frequency of hypoglycemia in patients with anterior pituitary insufficiency (43). Sometimes it is an outstanding symptom. We should emphasize that these patients are most sensitive to insulin and that if an insulin tolerance test is attempted, the patient should *not* be given 0.1 unit of insulin per kilogram of body weight—this amount has proved fatal.

A 45-year-old white woman registered at the Mayo Clinic in November, 1949. Her chief symptoms were spells of confusion and unconsciousness that occurred from two to four hours after meals and early in the morning. She also presented associated symptoms of adrenal insufficiency. In 1936, following a normal delivery, she had had a severe postpartum hemorrhage with shock and coma. Since that time she had never felt well. On physical examination, she was pale and had a waxy countenance and a loss of axillary and pubic hair. The value for blood sugar determined during one of her attacks in the hospital, was 25 mg. per 100 cc. An insulin tolerance test, using 0.03 unit of regular insulin per kilogram of body weight (a total of 1.5 units), was performed. The value for fasting blood glucose was 82 mg., the 15-minute value was 48 mg., the 30-minute value 30 mg., the 45-minute value 38 mg., the 60-minute value 40 mg., the 90-minute value 50 mg., the 120-minute value 51 mg., and the 180-minute value 63 mg. per 100 cc. Other laboratory tests confirmed the diagnosis of anterior pituitary insufficiency with secondary adrenal cortical insufficiency (Sheehan's syndrome).

Sheehan and Summers (44) reported that the glucose tolerance test is of no value in the diagnosis of hypoglycemia due to anterior pituitary deficiency.

Treatment of the adrenal cortical and anterior pituitary insufficiency of these patients by "end organ" replacement therapy prevents further attacks of hypoglycemia. The use of cortisone has been of greatest value.

Hypoglycemia has been an occasional finding in our cases of Addison's disease. The degree of hypoglycemia seems related to the degree of destruction of the adrenal cortices. Hypoglycemia as the only symptom in adrenal insufficiency has been reported by Rushton and co-workers (45). In their case, exploration for pancreatic adenoma was carried out. The patient died postoperatively of acute adrenal insufficiency. At postmortem examination, bilateral atrophy of the cortices of the adrenal glands was found. In retrospect, there had been one clue to the correct diagnosis and that was the patient's marked sensitivity to insulin, a characteristic of Addison's disease.

FUNCTIONAL HYPOGLYCEMIA

The most common type of spontaneous hypoglycemia is that known as functional hypoglycemia. Other terms used to describe this condition are physiologic hypoglycemia, functional hyperinsulinism, and neurogenic hypoglycemia.

Rennie and Howard (46) and Martin and Hellmuth (47) have pointed out that the diagnosis of functional hypoglycemia often has been made on inadequate grounds because of symptoms suggestive of hypoglycemia. The criteria for the diagnosis of functional hypoglycemia have best been defined by Conn (48).

We reviewed the records of 77 patients who were referred to us with the diagnosis of functional hypoglycemia. We discarded 44 of these at once; these patients were suffering from characteristic hyperventilation syndrome or frankly functional complaints of one sort or another, such as nervous exhaustion. Seventeen of the remaining 33 patients did have hypoglycemia but it was only one facet of the patients' problems. Each of these 17 patients had many frankly functional or psychosomatic complaints *plus* hypoglycemia. We refuse to believe that the hypoglycemia was causative; it was simply a part of the picture. The other 16 patients had many complaints which sounded as though they were related to the hypoglycemia per se, and most of these patients could meet Conn's criteria. But we still are careful in making this diagnosis. An example is the patient whose record we presented before the combined meeting of the American Diabetes Association and the Endocrine Society in Atlantic City in June, 1951.

A 35-year-old physician who was seen at the Mayo Clinic in 1948 had had, for six years, attacks of weakness, hunger, sweating, vertigo and trembling that occurred two hours after meals and that were immediately relieved by food. This condition began when he was under tension in the Army. There were no other symptoms. A glucose tolerance test revealed a value of 88 mg. for fasting blood glucose, a half-hour value of 128 mg., a two-hour value of 103 mg., a two-and-a-half-hour value of 70 mg., and a four-hour value of 48 mg. per 100 cc. Treatment with a high-protein, moderate-fat, and low-carbo-

hydrate diet gave complete relief of symptoms. This patient found he could produce a recurrence of his attacks by going on a high-carbohydrate diet.

In the fall of 1951 he returned to the clinic and told Dr. Grace Roth that he suspected his attacks were related to a pheochromocytoma. Her tests substantiated this diagnosis and Dr. Waltman Walters removed the tumor. In a recent communication the patient reported that his condition is improved. He has continued to follow the high-protein, low-carbohydrate diet but plans to study this aspect of his problem at a later date.

In functional hypoglycemia (48) there is a periodicity in the occurrence of symptoms, due to an increasing and decreasing state of emotional tension. The symptoms may last only five to thirty minutes, so that it may be difficult to get a determination of blood sugar during an attack. This condition may be present for years; 1 patient in our series had symptoms for twenty-five years. The symptoms rarely change in character from one year to the next, in contrast to true hyperinsulinism, which often gradually becomes more severe. Exercise may aggravate the symptoms of functional hypoglycemia, as well as those of organic hyperinsulinism. The symptoms of the former are of a milder degree than those due to true hyperinsulinism and to other types of organic hypoglycemia. The administration of glucose will relieve these symptoms promptly and completely and is an important diagnostic criterion, as other functional conditions such as nervous exhaustion are not relieved or are only partially relieved by glucose. Keating and Wilder (17) have stressed the fact that, in contrast to those of hyperinsulinism, the symptoms in functional hypoglycemia do not occur at night or before breakfast. The values for blood sugar during the attacks usually are not less than 50 to 60 mg., and in all the cases in our series they were greater than 50 mg., as contrasted to the very low readings in hyperinsulinism. However, if a glucose tolerance test is performed, it may be found that the concentration of blood sugar decreases to a value that is between 35 and 50 mg. in the third and fourth hours of the test. It has been our experience that, even with the foregoing criteria in mind, it may be difficult in some cases to distinguish between the symptoms of functional hypoglycemia and those due to psychoneurosis.

Conn (48) has expressed the belief that the diagnosis of functional hypoglycemia may be substantiated by use of the glucose tolerance test. He has stressed that the patient must be given a high-carbohydrate diet for at least three days prior to the test. In functional hypoglycemia the values for blood sugar dip sharply during the second to fourth hour of the test, often associated with the typical symptoms of mild hypoglycemia. This type of curve was found in only 20 per cent of the cases observed by Martin and Hellmuth (47). One of us (Skillern) has recently seen 2 patients whose values for fasting blood sugar were normal, the typical diabetic glucose tolerance curves falling to hypoglycemic levels with the production

of symptoms during the fourth and fifth hours of the tests. Both of these patients had had, for more than fifteen years, the classic symptoms of functional hypoglycemia, which were reproduced during the tests. Neither one of them had a family history of diabetes. Both were moderately obese males. They had had a high carbohydrate intake prior to the tests. Both were free of symptoms on a high-protein, low-carbohydrate diet. We suspect that these diabetic glucose tolerance curves may be indicative of a latent diabetes, rather than having any relation to the functional hypoglycemia. McCullagh (49) has observed clinical diabetes developing several months later in a patient who previously was found to have this type of glucose tolerance curve. Rennie and Howard (46) have observed the occurrence of hypoglycemic glucose tolerance curves in the case of normal persons without symptoms of hypoglycemia. We have also seen this occur frequently with and without symptoms of hypoglycemia in emotionally labile or tense people, in the absence of a previous history of functional hypoglycemic symptoms. Therefore, it is our belief that the diagnosis of functional hypoglycemia should be made only if a typical history of episodes such as those just described are present in an emotionally labile individual, and if the hypoglycemia and symptoms are reproduced during a glucose tolerance test.

There is another type of functional hypoglycemia seen in patients who have undergone gastroenterostomy, partial gastrectomy or total gastrectomy. The symptoms are essentially the same and occur at the same time as those described for functional hypoglycemia in those patients who have not had a gastric operation. This syndrome occurs in only a small percentage of patients who have undergone an operation on the stomach. These patients are also tense and emotionally labile. The hypoglycemic syndrome in such patients should be carefully distinguished from the dumping syndrome, which occurs immediately after eating rather than two to four hours postprandially. The glucose tolerance curve differs from that observed for the patient with an intact stomach, in that there is an initial hyperglycemia to a diabetic level with a subsequent sharp decrease in the concentration of blood sugar. Since most patients who have had a gastric operation show this type of curve, the history must be carefully correlated with the degree of hypoglycemia and resultant symptoms produced during the glucose tolerance test.

Conn (48) has discussed the treatment of functional hypoglycemia and has described the use of a diet high in protein and low in carbohydrate. The content of this diet varies from 120 to 140 Gm. of protein and from 50 to 100 Gm. of carbohydrate. These patients, as we have pointed out, are usually tense or psychoneurotic individuals and are often in need of

"amateur" or professional psychotherapy. Sedation may be of definite help.

OTHER TYPES OF HYPOGLYCEMIA

We shall not discuss any of the other types of hypoglycemia mentioned in Table 1. We should like to conclude by saying that sometimes hypoglycemia that is difficult to explain and that disappears spontaneously is seen in children. Sisson (50) stated that the symptoms presented by the infants he observed varied greatly and were caused by many conditions. Cerebral anoxia with involvement of the respiratory centers appeared to be the cause of many of these symptoms. In the 4 instances of idiopathic hypoglycemia observed by him, the children's ages varied from 18 months to 5 years. Three of the patients underwent exploration of the pancreas for islet cell tumor, with negative results; the fourth did not undergo exploration. Further attacks of hypoglycemia were prevented by giving a low-carbohydrate and high-protein diet. Follow-up reports on 3 of these patients, in 1 of whom the attacks were reported by John (51) as controlled by diet alone, indicate that they were completely asymptomatic one to two years later. The fourth continued to have attacks. The cause of these hypoglycemic reactions could not be ascertained, but it is our impression that hypoglycemic reactions that occur in young children are much less likely to be due to functioning islet cell tumors than are those that occur in the adult.

Recently, McQuarrie and co-workers (52) reported the occurrence of hypoglycemia of the idiopathic type in 2 children in one family and in 3 siblings of another family. This type of hypoglycemia is of hereditary origin and is found early in life. It is thought to be due to an absence of the alpha cells in the islets of Langerhans, as demonstrated by McQuarrie and his co-workers (53), which normally supposedly exert a hyperglycemic effect. Adrenocorticotropic hormone was found to be highly effective in controlling the hypoglycemia in these children, as long as it was administered.

SUMMARY

In this discussion there has been an attempt to emphasize the importance of separating hyperinsulinism from all other types of hypoglycemia. If hyperinsulinism is suspected and diagnosed, the patient's life may be saved by surgical treatment. If spontaneous hypoglycemia has been erroneously diagnosed, the physician may aid many patients by disproving the diagnosis; if spontaneous hypoglycemia is proved to be present, the patient may be benefited by following the program advised by Conn.

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Teaching Clinic

USE OF THE INTRAVENOUS ACTH TEST IN CLINICAL PRACTICE

"THE leading and characteristic features of the morbid state to which I would direct attention are anaemia, general languor and debility, remarkable feebleness of the heart's action, irritability of the stomach, and a peculiar change of colour in the skin, occurring in connexion with a diseased condition of the 'supra-renal capsules.' " Since 1855, when Addison thus described the disease which now bears his name (1), other features have been noted regularly in patients suffering from insufficiency of the adrenal cortex: poor tolerance for fasting, with a tendency toward a low blood sugar concentration; difficulty in retaining salt within the body, so that withdrawal of salt from the diet is also poorly tolerated; and difficulty or delay in excreting water when a large amount is ingested. Though Addison's disease remains relatively rare, increasing interest in the adrenal glands, as well as great improvement in the treatment of adrenal insufficiency make accurate diagnosis of adrenal disease increasingly important.

Unfortunately, none of the features just mentioned can be considered specific for Addison's disease alone. Although the various diagnostic procedures based upon the metabolic defects listed have been of considerable help in establishing the diagnosis of adrenal cortical insufficiency, the great advantage of being able to use a specific adrenocorticotropic hormone in the measurement of adrenal cortical function is obvious. Tests based on intramuscular administration of ACTH (2, 3) have proven of great value, but their usefulness has been limited by a significant loss of activity suffered by some preparations of ACTH when injected intramuscularly (4). Experience has shown that this difficulty can be eliminated by *intravenous* administration of ACTH. Furthermore, quantitative studies of adrenocortical activation have demonstrated that the continuous intravenous infusion of 15–20 units of ACTH over an eight-hour period produces near-maximal stimulation of the adrenal cortex in the normal subject (5).

The recent development of long-acting preparations of highly purified ACTH in a gelatin vehicle would appear to have overcome the problem of intramuscular inactivation. The daily injection of 20–40 *U.S.P. units* of ACTH in gelatin produces adrenal cortical activation which is equal to, or even greater than, that resulting from the eight-hour intravenous infusion of an equivalent dose. It is apparent that the route of ACTH ad-

ministration is not of primary importance so long as the adrenal cortical stimulation produced is intense. Since previous quantitative studies (5) have demonstrated that the duration of stimulation exerts a major influence on the ultimate intensity of adrenal response, intramuscularly injected preparations of ACTH capable of exerting a protracted action should provide an adequate stimulation. Thus far, no significant degree of intramuscular inactivation or "ACTH-resistance" has been encountered with these highly purified materials.

In performing the intravenous ACTH test, the blood eosinophil count and the urinary excretion of 17-ketosteroids and 17-hydroxycorticoids are used as indices of the adrenal response. The latter procedure (6) has considerably increased both the facility and the precision of the test. After a "control" 24-hour urine collection for steroids, ACTH in a dose of 20–25 U.S.P. units is administered by intravenous drip over a period of eight hours; the ACTH is dissolved in 500 ml. of normal saline solution. Blood is drawn for eosinophil counts immediately before, and from eight to ten hours after, the start of the infusion. A second specimen of urine is collected for twenty-four hours, including the period of the infusion, and is analyzed for steroids. Since the urine collections were made on the general medical wards, the determinations of 17-hydroxycorticoids and 17-ketosteroids presented here have been corrected according to the mean daily creatinine excretion over the period of the test, to eliminate the possible error introduced by incomplete collections.

In the overwhelming majority of patients it has been found (7) that adrenocortical stimulation by ACTH on two successive days constitutes an adequate test. With the occasional difficult diagnostic problem a three-day testing period provides a more reliable indication of the presence or absence of functioning adrenal cortical tissue. This is particularly applicable to patients with long-standing adrenal cortical atrophy secondary to hypopituitarism, in whom evidence of an adrenal response may occasionally be deferred until the third day of the test. In such circumstances, repetition of the ACTH infusions for an additional two or three days may elicit a more pronounced adrenal response. Untoward reactions to intravenous ACTH, in the form of fever or urticaria, have been rare and in our experience have occurred exclusively in patients with adrenal insufficiency, primary or cortisone-induced. We have otherwise not encountered a serious reaction to ACTH administered according to the technique described, and with the highly purified ACTH preparations now available, sensitivity reactions appear to be a negligible risk.

The response to the first eight-hour ACTH infusion, in *normal subjects and in hospital patients without adrenal disease* includes a fall in eosinophils of 85 per cent or more and a mean rise in urinary 17-ketosteroids of 4.4 mg.

per twenty-four hours (7). The range of eosinophil falls in 100 such subjects was 80 to 100 per cent and only 6 patients showed a fall of less than 90 per cent. The range of increments in 17-ketosteroid excretion, however, was very wide (0.9 to 19 mg. per twenty-four hours) and approximately 10 per cent of hospital patients showed little or no rise in ketosteroids with the first day's infusion. With a second day of infusion, however, an increase in ketosteroid excretion was uniformly observed. In 35 subjects there was a mean rise of 8.7 mg. (range, 4.7 to 16.0) above the control level on the second day of ACTH administration. It may be seen that the second infusion leads to a much more clear-cut result than a single day's infusion. Preliminary experience with the determination of urinary 17-hydroxycorticoids shows a rise of about 10 to 20 mg. on the first day of ACTH, with a further rise of 5 to 10 mg. on the second day. In 35 patients with *Addison's disease* the eosinophil fall was 30 per cent or less even with repeated ACTH stimulation (range, −30 to +40 per cent) and the change in ketosteroid excretion was insignificant (range, −2.9 to +1.9 mg. per twenty-four hours). There has been no significant change observed to date in the 17-hydroxycorticoid excretion of patients with Addison's disease on repeated ACTH stimulation. In *hypopituitarism* there is either a subnormal response to the first day of ACTH infusion or no response at all; with repeated infusion of ACTH, however, the various indices of adrenal function show an increasing degree of response, often approaching normal values by the third day.

Among the clinical situations in which the intravenous ACTH test has proved to be of the greatest value are the following: 1) elimination of the diagnosis of Addison's disease in patients with suggestive signs or symptoms, including weakness and fatigability, weight loss, gastrointestinal dysfunction, or pigmentation; 2) detection of adrenal insufficiency, often of acute onset, as a masked complication of surgical trauma or other severe stress, and 3) differentiation of secondary adrenal insufficiency (hypopituitarism) from the primary condition. To illustrate the value of the ACTH test in these important clinical problems, 3 case histories are presented.

CASE HISTORIES

Case I. Normal ACTH response in a patient with symptoms suggesting Addison's disease

Mrs. D. C. (*P.B.B.H. 7E316*), a 21-year-old housewife, was admitted to the Peter Bent Brigham Hospital for study because of a diagnosis of Addison's disease made at another hospital.

One year prior to admission she had noted the onset of weakness, lethargy, anorexia and nausea, but no vomiting. At the same time she began to be troubled by menorrhagia and metrorrhagia. These menstrual difficulties were treated successfully with an unknown hormone preparation. Concomitantly with the development of the above

symptoms, the patient noticed darkening of her gums and of the skin under her eyes and around her waist. She lost 20 pounds in weight over a period of six months. There were occasional muscular cramps in her legs. She ate as many as six candy bars in a day but experienced no salt craving. The diagnosis of Addison's disease was made in another hospital on the basis of the clinical picture, a low serum chloride level (90 mEq. per liter) and a small heart by x-ray examination. She was treated with oral cortisone, the implantation of one 125 mg. pellet of desoxycorticosterone acetate, and 3 to 4 grams of supplementary salt per day. The patient stated that she did very well for two or three months, on this regimen, with a gain of approximately 7 pounds in weight and with diminution of nausea and of pigmentation of the gums. In the month prior to admission, however, nausea and weakness returned, together with a slight weight loss, in spite of continued therapy. The past history was unremarkable except for menstrual irregularity and some emotional maladjustment related to marriage.

On physical examination the temperature, pulse and respiration were normal; blood pressure was 100/60. The patient was a small but well developed young woman, who seemed comfortable. There was diffuse brownish pigmentation of the skin, with accentuated pigmentation around the eyes, in the pellet implantation scar on the back and in the abdominal folds. Dark freckles were sparsely scattered over the body. Pressure points were not pigmented, nor were the areolae of the breasts. Two healed abdominal scars were not pigmented, and there was no increased pigmentation of the perineum or genitalia. Axillary and pubic hair were normal. The mouth revealed brownish pigmentation along both alveolar ridges and in the median raphe of the palate. The heart and lungs appeared to be normal and the rest of the examination revealed no abnormal findings.

Laboratory data: The hematocrit was 40 per cent, and the white blood count, blood smear and differential count were normal. The level of fasting blood sugar was 80 mg., of blood urea nitrogen 6 mg., and of serum cholesterol 170 mg. per 100 ml. Serum sodium concentration was 142, potassium 4, and chloride 111 mEq./liter. The B.M.R. was −5 per cent. X-ray studies revealed a small heart, clear lung fields and no suprarenal calcification. An electroencephalogram was within normal limits.

During studies in the hospital all adrenocortical replacement therapy was discontinued, the patient being maintained on a hospital diet without supplementary salt. An eight-hour intravenous infusion of normal saline containing 20 U.S.P. units of ACTH was given on three consecutive days (Fig. 1). Steroid excretion on the control day was within normal limits. ACTH infusion led to normal eosinophil falls and an immediate and progressive increase in 17-ketosteroid and 17-hydroxycorticoid excretion.

These results established the presence of good adrenal cortical function. The patient was discharged without hormone therapy and in the subsequent six months she has remained in good health. Three recent determinations of urinary 17-ketosteroids have been within normal limits. The final diagnosis is normal adrenal cortical function.

Case II. Acute adrenal insufficiency as a masked complication of surgery

Mr. C. S. (*P.B.B.H. 8B660*), a 44-year-old dress designer, was under treatment for chronic duodenal ulcer in another hospital when a radio-opaque mass was found by intravenous pyelography at the lower pole of the right kidney. The kidney was removed, together with the right adrenal gland, and a well encapsulated renal-cell carcinoma was found in the renal parenchyma. From the third postoperative day the patient experienced "black-outs," with profuse sweating, flushes, and weakness; these were associated with hypochloremia and were relieved by intravenous infusions of 5 per cent glucose

in normal saline. He improved sufficiently to be discharged from the hospital on the twelfth day after the operation. Ten days later, however, he was re-admitted because of recurrence of the "black-outs," now accompanied by cramp-like abdominal pains, blurring of vision, dizziness and anorexia. Chemical determinations revealed the following concentrations: blood nonprotein nitrogen, 69 mg. per 100 ml.; serum sodium 126, serum potassium 6.7, and serum chloride 100 mEq./liter. These findings, together with the relief of symptoms by intravenous and oral administration of sodium chloride,

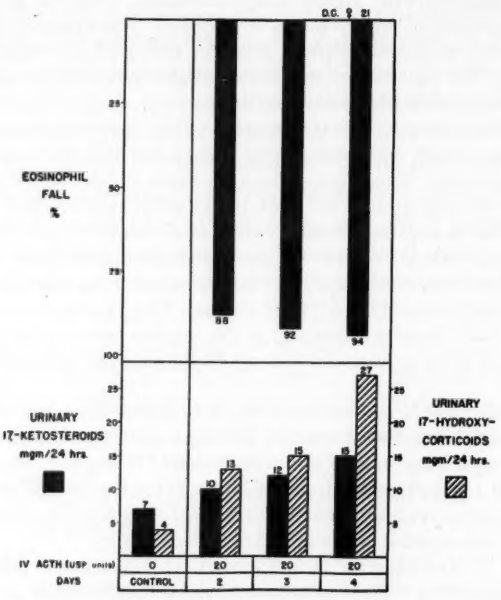

FIG. 1. Response to intravenous ACTH in Case I.

suggested acute adrenal insufficiency as a possible diagnosis. The patient was transferred to the Peter Bent Brigham Hospital.

On physical examination he was found to have a blood pressure of 90/64 mm. Hg and abnormal pigmentation limited to the suture marks of the recent nephrectomy scar. By x-ray examination the heart size was 4 per cent below the predicted average. Atelectasis was observed at the base of the right lung, but no abnormalities were found in a flat x-ray film of the abdomen or in a barium meal with follow-through.

Laboratory data: The hematocrit was 43 per cent; the white blood count and blood smear were not abnormal except for an eosinophilia of 12 per cent. Chemical analyses showed the following concentrations: fasting blood sugar, 70 mg., and blood urea nitrogen 38 mg. per 100 ml.; serum sodium 136, serum potassium 6.8 and serum chloride 94 mEq. per liter; serum CO_2 19.6 mM. per liter.

ACTH (20 U.S.P. units) was administered intravenously over an eight-hour period on three successive days. The results are shown in Figure 2. Since there were virtually no change in circulating eosinophils and no significant increase in 17-ketosteroid excre-

tion during three days of ACTH stimulation, a diagnosis of primary adrenal insufficiency was made. Determination of urinary 17-hydroxycorticoids was not carried out at this time.

The patient was given cortisone, 25 mg. a day by mouth, and desoxycorticosterone acetate in oil, 2.5 mg. intramuscularly each day, with a resultant increase in body weight of 4 pounds and an increase in blood pressure from 98/60 to 120/84 mm. Hg in six days. He was discharged on a normal diet with unrestricted salt. Recently, a long-acting form of desoxycorticosterone (desoxycorticosterone trimethylacetate) has been substituted

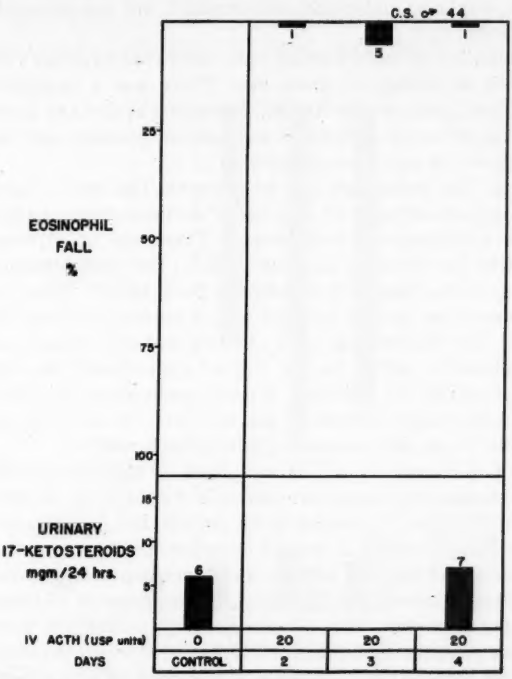

FIG. 2. Response to intravenous ACTH in Case II. Urinary steroids were not measured on days 2 and 3.

for the daily injections of desoxycorticosterone. He continues to do well at the present time, nineteen months after the diagnosis of adrenal insufficiency was first made. Further tests during three subsequent hospital admissions have shown no adrenal response to ACTH. The final diagnosis is primary adrenal cortical insufficiency.

Case III. Differentiation of secondary adrenal insufficiency (hypopituitarism) from Addison's disease

Mrs. E. I. (*P.B.B.H. 6E821*), a 60-year-old housewife, entered the Peter Bent Brigham Hospital for evaluation of her endocrine status.

At 28 years of age she had had a stillborn infant, delivered prematurely, following

one week of profuse vaginal bleeding. Menses recommenced, only to stop suddenly two years later. There was subsequent loss of axillary and pubic hair. When she was 43 years old she was found to have diabetes mellitus, and treatment with insulin was instituted. In the same year she was given 30 mg. of desiccated thyroid daily because of a persistently low basal metabolic rate. At the age of 59 glycosuria was said to have disappeared, and the administration of insulin was discontinued; she continued to take 30 mg. of desiccated thyroid daily. A few months later she was hospitalized elsewhere because of fainting spells, weakness and anorexia. Blood sugar levels were found to be normal without insulin, and the B.M.R. was −33 per cent. Vague gastrointestinal distress, nervousness, weakness and weight loss persisted, and she was sent to this hospital because of these complaints.

Physical examination revealed a small, pale, somewhat bald but youthful-appearing white woman, with no axillary or pubic hair. There was a bradycardia of 52 beats per minute; the blood pressure was 100/50. The skin was dry and atrophic; there were kraurosis and advanced senile atrophy of the external genitalia and an atrophic cervix and uterus were found on pelvic examination.

Laboratory data: The hematocrit was 44 per cent. The level of fasting blood sugar was 76 mg., of blood urea nitrogen 27 mg., and of serum cholesterol 433 mg. per 100 ml.; serum electrolyte concentrations were normal. There was no glycosuria. An electrocardiogram revealed low T-waves and low E.M.F.; the electroencephalogram showed predominating waves of a frequency slightly less than normal. X-ray studies revealed a 15 per cent decrease from normal in heart size, a normal pituitary fossa, and normal bone architecture. The B.M.R. was −14 per cent and the 24-hour accumulation of a tracer dose of radioactive iodine by the thyroid gland was 0 per cent; these results may have been modified by previous thyroid medication. An intravenous glucose tolerance test showed a slight decrease in glucose tolerance, and a fall in the serum phosphorus level of only 10 per cent, consistent with diabetes mellitus.

A dose of 20 U.S.P. units of ACTH was given by eight-hour intravenous infusion on three successive days; the results are shown in Figure 3. On the first day of ACTH stimulation there was a fall in circulating eosinophils, but it was subnormal in degree; there was no significant increase in steroid excretion. On the second and third days of of ACTH, the eosinophil fall was normal, and there was a substantial increase in 17-hydroxycorticoid excretion on the third day; the response in 17-ketosteroid excretion must be considered equivocal. This combination of findings was interpreted as being consistent with the presence of adrenal cortical tissue, of which the response was delayed because of previous "inactivity." Therefore, this pattern of adrenal cortical response to ACTH provided confirmatory evidence for a diagnosis of panhypopituitarism.

The patient was discharged on 25 mg. of cortisone acetate and 30 mg. of desiccated thyroid by mouth daily, 50 mg. of testosterone propionate weekly by intramuscular injection, and 15 mg. of desoxycorticosterone trimethylacetate monthly by intramuscular injection. There has been a decided symptomatic improvement since discharge; she shows a high normal fasting blood sugar level with occasional glycosuria at mid-day. The final diagnosis is adrenal cortical insufficiency secondary to hypopituitarism.

DISCUSSION

The cases presented have been chosen as examples of the types of adrenal response to intravenous ACTH commonly seen in clinical practice. Case I showed a prompt and progressive response to ACTH; this is the

normal pattern. Case II showed no response on three successive days of ACTH administration; this is characteristic of primary adrenal insufficiency. Case III presented a response that would seem to fall midway between those of Cases I and II; the eosinophil fall was subnormal on the first day of ACTH administration, but greater than that seen in Addison's disease, and the 17-hydroxycorticoid and 17-ketosteroid determinations revealed a delayed and subnormal increase during the testing period. It is apparent that the rapidity and intensity of the adrenocortical response to ACTH in hypopituitarism is variable, depending on the duration and extent of adrenal cortical atrophy. In most such cases a normal degree of

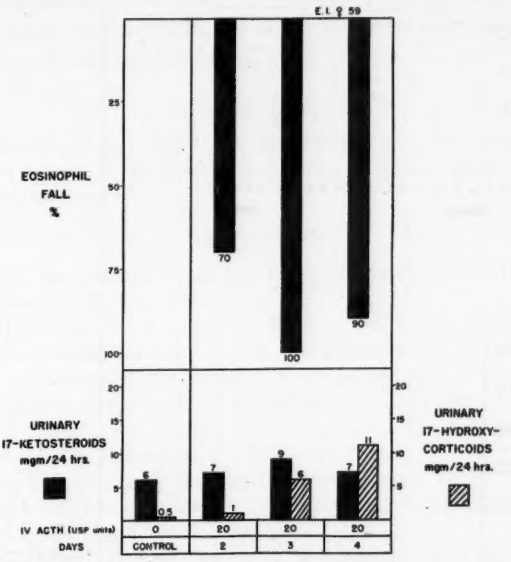

FIG. 3. Response to intravenous ACTH in Case III.

eosinopenia and a significant rise in urinary steroid excretion occur after three days of intravenous infusions of ACTH. In occasional cases, however, a more prolonged period of stimulation is necessary to produce a satisfactory adrenal response.

The eosinophil count remains a very informative yet simple procedure, when used in conjunction with intravenous ACTH. In order to provide a reliable index of adrenal cortical activation it is essential that control eosinophil counts be sufficiently high (above 100 cells per cu. mm.) to prevent misinterpretations of changes resulting from the intrinsic error of chamber counting. It is to be emphasized, however, that a normal re-

sponse to the intense adrenal stimulation produced by prolonged ACTH infusions exceeds the changes in eosinophil level due to normal diurnal variation. Furthermore, no overlap between the eosinopenia of normal subjects and that of patients with Addison's disease has been encountered (Fig. 4). Indeed, an interpretation of the intravenous ACTH test based on changes in circulating eosinophils alone would be accurate in the great majority of cases. In those instances in which the initial response is intermediate (*e.g.*, Case III), or when control eosinophil counts are too low for accurate interpretation, urinary steroid determinations are essential.

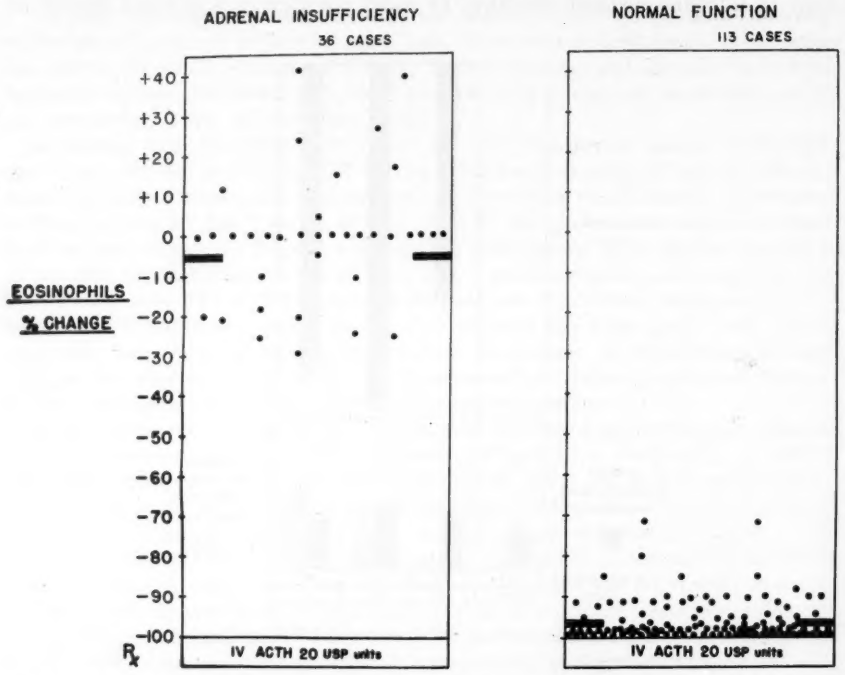

FIG. 4. ACTH was infused intravenously over an eight-hour period. Eosinophils were counted immediately prior to, and eight to ten hours after, beginning the infusion. The transverse bars represent mean eosinophil falls.

The estimation of urinary 17-hydroxycorticoids is a most sensitive index of adrenal function and, as these cases illustrate, appears to give results more conclusive than those given by 17-ketosteroid estimations. Moreover, the measurement of urinary 17-hydroxycorticoids is, in contrast to changes in the level of circulating eosinophils, a more *direct* measure of adrenal cortical secretory activity and therefore constitutes the best available index of adrenal responsiveness to ACTH.

SUMMARY

Three cases are presented to illustrate the clinical use and interpretation of the intravenous ACTH test. The first patient, whose clinical picture simulated Addison's disease, showed a prompt, normal response to ACTH. The second patient, who collapsed following major surgery, was shown to have no adrenal response to ACTH, allowing a diagnosis of adrenal insufficiency to be made promptly and before most of the classic features of Addison's disease had developed. The third patient, with evidence of multiple endocrine deficiencies, was shown to have a "delayed normal" response to ACTH, consistent with anterior pituitary deficiency. The eosinophil count and the urinary excretion of 17-hydroxycorticoids appear to be the most useful indices of adrenal function in conjunction with the intravenous administration of ACTH.

Acknowledgments

The authors would like to thank Dr. A. H. Holland, Jr., Armour Laboratories, Chicago, Illinois, and Dr. H. F. Hailman, The Upjohn Company, Kalamazoo, Michigan, for the ACTH used in these studies.

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Editorial

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MEASUREMENT OF ADRENAL CORTICAL FUNCTION

EASY fatigability, weakness and asthenia constitute presenting symptoms in a considerable proportion of the patients seen by the general practitioner, internist and endocrine-metabolic specialist. Adrenal cortical insufficiency must be considered in the differential diagnosis of the problem presented by such patients, although many with these complaints exhibit important psychologic and emotional aspects. In patients presenting these symptoms in conjunction with serious infections, surgical operations and traumatic injuries, the problem frequently arises as to whether or not adrenal cortical response is adequate or optimal in the presence of severe stress. The availability and efficacy of adrenal cortical hormone therapy, and the reassurance which can be given to a patient with intact, adequately functioning adrenals, justify more general application of effective diagnostic "screening" procedures. Of all the hormonal estimations available to the practicing physician, none is more specific than the estimation of adrenal cortical response to exogenously administered ACTH.

The level of spontaneous adrenal cortical activity should be measured whenever time permits and suitable facilities exist. The simplest method of analysis is to determine the 24-hour urinary excretion of 17-hydroxycorticoids. Standardized technics for these determinations have been greatly improved in recent months and many laboratories are now equipped to make these measurements. Normal steroid values in the absence of undue stress, or elevated corticoid values in the presence of serious illness, exclude significant adrenal cortical insufficiency as an important contributing factor to symptomatology or clinical condition. Low values for steroid

excretion, on the other hand, suggest inadequate adrenal cortical response and warrant definitive tests for primary or secondary adrenal cortical insufficiency. This condition may be transient or permanent. The 17-ketosteroid excretion does not always follow the elevation of 17-hydroxycorticoid excretion; hence normal values for 17-ketosteroids do not exclude increased adrenal secretory activity.

Primary adrenal cortical insufficiency is most specifically determined by evaluating the response of the adrenal cortex to exogenously administered ACTH. A rapid screening procedure utilizes the percentile fall in the level of circulating eosinophils four hours following the intramuscular administration of ACTH. *Epinephrine should not be employed* for this purpose as a substitute for ACTH, as explained later. More definitive evaluation of the adrenal capacity to respond may be obtained from determination of the change in urinary steroid response following the *intravenous* administration of ACTH. For this purpose, a control 24-hour excretion of steroid is essential. Following the control collection, an eight-hour continuous intravenous infusion of 20–25 units of ACTH is given in 500 ml. of saline or glucose solution. Eosinophil determinations may be made at zero hour, six hours, and ten hours (two hours following the termination of the infusion). The urine specimen is collected for twenty-four hours (including the eight-hour period of infusion) and analyzed for 17-hydroxycorticoids. A normal response to the eight-hour infusion includes a fall in eosinophils of at least 85 per cent at the ten-hour point, and a rise in total 17-hydroxycorticoid excretion of 10–15 mg. Failure to observe a response suggests the need for daily eight-hour infusions of ACTH for at least two or three successive days.

Secondary adrenal cortical insufficiency should be suspected in the presence of a low initial value for urinary steroids, or a subnormal response to the intravenous administration of ACTH. In the presence of pituitary insufficiency with secondary adrenal cortical insufficiency, one would anticipate a slow but progressive increase in the degree of eosinophil response and steroid excretion values following successive daily eight-hour intravenous infusions of ACTH. It may require several days to attain levels compatible with a normal response. However, the curve of response—that is, the successive daily increment—provides highly suggestive evidence of the condition.

Unfortunately, in man there is no evidence that the administration of epinephrine is capable of eliciting a measurable increase in the urinary excretion of 17-ketosteroids or 17-hydroxycorticoids. Thus, the response of the adrenal cortex to ACTH on the one hand and its failure to respond to epinephrine on the other, do not constitute a valid mechanism for differentiating primary and secondary adrenal cortical insufficiency.

Furthermore, recent studies in completely bilaterally adrenalectomized man substantiate earlier observations on patients with Addison's disease that epinephrine is capable of inducing an eosinopenia in the absence of functioning adrenal cortical tissue.

Adrenal cortical insufficiency secondary to the administration of cortisone and hydrocortisone (compound F) is becoming an increasingly important clinical consideration. Following prolonged administration of these hormones, adrenal involution of varying degrees of intensity may be anticipated. Adrenal insufficiency produced by this mechanism may be detected by the standardized four-hour ACTH test, as previously described. However, to restore adrenal cortical function to a normal level may require intense stimulation for a prolonged period. For this purpose it is most practical to give an eight-hour intravenous infusion of 20–25 units of ACTH on at least three successive days. Again, the differentiation between primary adrenal insufficiency and insufficiency secondary to pituitary inhibition is based upon the progressive response of the gland to successive therapeutic doses of ACTH.

SUMMARY

The response of the adrenal cortex to exogenously administered ACTH is one of the most specific and predictable hormonal measurements available. Occasionally, ACTH given intramuscularly is inactivated or destroyed and hence does not reach its target gland—the cortex. Following the intravenous administration of ACTH, this almost never occurs. The response of the circulating eosinophils to ACTH is highly specific as an indication of increased adrenal secretion of 11,17-oxysteroids; this is not true for epinephrine. The measurement of 17-ketosteroid and 11,17-oxysteroid excretion following ACTH administration increases the specificity of the interpretation of the ACTH response and adds an important quantitative evaluation. At present, there is no specific differentiating test for pituitary ACTH deficiency. Progressive increased responsiveness of the adrenal cortex, beginning from a subnormal level, is, however, highly suggestive of either primary, spontaneous pituitary insufficiency or adrenal atrophy secondary to adrenal steroid therapy.

GEORGE W. THORN, M.D.

Questions and Answers

(Questions submitted at the 1952 Postgraduate Assembly of the Endocrine Society.)

Answers by Lawson Wilkins, Baltimore, Md.

QUESTION: I have a 27-year-old female with surgically proven adrenal hyperplasia producing an adrenogenital syndrome. The onset of symptoms was at age 12. The urinary 17-ketosteroids were 60 mg. in twenty-four hours, and after two weeks of 100 mg. of cortisone daily by mouth, they rose to 68 mg. There was no symptomatic improvement. What is the treatment of choice?

ANSWER: In cases of the adrenogenital syndrome due to adrenal hyperplasia I have never known an instance in which cortisone failed to suppress the adrenal hyperactivity and reduce markedly the urinary 17-ketosteroids.

If this were my patient I would administer 50 mg. cortisone *intramuscularly* each day for ten days, as the effects of cortisone are more predictable when given by the intramuscular rather than by the oral route. If the 17-ketosteroids have not decreased to 10 mg. per day or less by the end of this time, I would again explore transperitoneally for a neoplasm. There are the following possibilities 1) There may be an adrenocortical tumor in an aberrant situation. We have reported one case in which the tumor was situated inside the liver. 2) There may be an "adrenal rest" tumor of the ovary. 3) Bilateral adrenal hyperplasia may possibly undergo a neoplastic change, with multiple areas of nodular hyperplasia. Under these conditions perhaps it can no longer be suppressed by cortisone. 4) The possibility of arrhenoblastoma of the ovary must be considered, although I know of no cases in which the urinary 17-ketosteroids were so high. 5) I assume that the patient has true adrenogenital syndrome as stated, and not Cushing's syndrome. The latter does not respond to cortisone.

I should like to hear more details concerning this case.

QUESTION: Could the fact that virilism continues following removal of 1½ adrenals mean that there is a compensatory hypertrophy (secondary to removal)? Are we dealing with a secondary condition, *i.e.*, is the virilism due to a secondary adrenal condition?

ANSWER: I believe that in the adrenogenital syndrome with bilateral adrenal hyperplasia the fundamental disturbance consists of disability of the adrenals to convert steroidal precursors to the normal adrenal hormones such as compound F, and a diversion of the pathway of synthesis toward the formation of steroids with androgenic properties. To supply the normal requirements for compound F, pituitary ACTH secretion is increased; adrenal hyperplasia results and still larger amounts of androgenic steroids are secreted. The removal of 1½ adrenals does not correct the fundamental disturbances. Excessive secretion of ACTH continues and the remaining adrenal hypertrophies and continues to manufacture abnormal steroids.

QUESTION: What is the treatment of choice in the case of children (especially girls) taller than usual, in whom there is a familial tendency to tall stature? These are indi-

viduals with normal body proportions and no evidence of pituitary dysfunction. They are around the age of puberty; no menarche has occurred, but they are beginning to show secondary sex characteristics.

ANSWER: I believe that it is wrong to attempt to alter constitutional and hereditary traits of body build by hormonal interference. Although theoretically the administration of estrogens might inhibit growth and hasten epiphyseal fusions, such treatment is bound to inhibit the secretion of pituitary follicle-stimulating hormone and interfere with the development of the normal pituitary-ovarian mechanism which occurs at puberty. Likewise one could not expect radiation of the pituitary to inhibit selectively the growth mechanism without destroying other important pituitary functions. The overenthusiastic and ignorant administration of potent endocrine preparations in normal constitutional variations of growth and development is to be greatly deplored!

Answer by Peter H. Forsham, San Francisco, Calif.

QUESTION: What is the evidence for the existence of two types of TSH?

ANSWER: M. A. Greer (*J. Clin. Endocrinol. & Metab.* **12**: 1259, 1952) working on rats has shown a dichotomy between the ability of the pituitary gland to maintain thyroid size and to stimulate thyroidal accumulation of iodine. When the pituitary was isolated from the hypothalamus, or certain areas of the anterior hypothalamus were destroyed by electrolytic lesions, the pituitary was unable to stimulate the growth of thyroid cells but nevertheless was able to maintain fairly normal thyroidal iodine metabolism. This led to the postulation of a growth factor and a metabolic factor, the former dependent upon direct contiguity of the hypothalamus for its secretion.

F. X. Gassner (personal communication) working on bovine pituitary protein fractions prepared by S. Steelman of Armour & Co. found that certain TSH preparations show high cell-growth-promoting capacity with little iodine discharge potential, whereas other TSH preparations show the reverse. B. Dobyns (unpublished work) has demonstrated chromatographically the existence of two distinct pituitary fractions, one producing marked exophthalmos in the Atlantic minnow, with little TSH activity as measured by iodine discharge from the gland or hypertrophy—distinct from a true TSH fraction. Reports on this recent work will demonstrate the existence of three separate pituitary factors, one involved in exophthalmos, one in cell-height regulation, and the other in thyroidal iodine metabolism.

Answers by Rulon W. Rawson, New York, N.Y.

QUESTION: What is the concept of the pathologic physiology of thyroid crisis?

ANSWER: The pathologic physiology of thyroid crisis is not well understood. It has been defined as a life-endangering augmentation of the symptoms of thyrotoxicosis, in which the patient's response is out of proportion to the exciting stimulus, whatever its nature (thyroidectomy, pneumonia, or wound sepsis.) The mechanism for the symptomatology recognized as thyroid storm, is not understood. It is of interest that several patients with thyroid storm have been successfully and even dramatically treated with a watery extract of the adrenal or with cortisone. Although the mechanism is likewise unexplained, this observation might possibly suggest adrenal failure as a part of the pathologic physiology of thyroid crisis.

QUESTION: What are the effects, if any, of cortisone on the exophthalmos and the enlargement of the thymus and lymph nodes found in Graves' disease?

ANSWER: Cortisone has been described by some investigators as having no effect on exophthalmos; other investigators have reported beneficial results. There are no data on the effect of cortisone on the enlargement of the thymus and lymph nodes in Graves' disease. Adequate treatment with cortisone has been observed to diminish the circulating lymphocytes.

QUESTION: What is the physio-pathologic explanation of localized myxedema associated with exophthalmos and signs of Graves' disease?

ANSWER: The pathologic physiology of localized myxedema is not any better understood than is the severe exophthalmos of Graves' disease. It has been reported that there is an increased level of mucoprotein not only at the site of localized myxedema, but also in the retro-orbital tissues. A similar increase in mucoprotein has been observed in the tissues of animals treated with pituitary extracts rich in thyrotropic hormone. Why this process is localized as it is, remains an enigma.

QUESTION: Please discuss the use of estrogens in the treatment of exophthalmos.

ANSWER: Estrogenic therapy in the treatment of exophthalmos has been advocated by various investigators. In the experience of the writer, it has been of no value.

QUESTION: Comment on enlargement of the thyroid without any other symptoms in young females in relation to the first menstrual period.

ANSWER: The enlargement of the thyroid in young females at the time of menarche suggests an increased need for thyroid hormone with the development of ovarian function. It may represent a simple accentuation of an iodine deficit. The writer's interpretation of such an enlargement is that the gland is hyperplastic and that it can be benefited by the administration of thyroid hormone. If the enlargement is allowed to progress, the picture of colloid goiter usually develops.

QUESTION: For how long after disappearance of symptoms do you maintain treatment with Tapazole or similar compounds in nonsurgical cases of toxic goiter?

ANSWER: The writer would advocate that Tapazole or similar antithyroid drugs be administered for at least one year after the symptoms of the disease have disappeared.

QUESTION: What is the treatment of thyrotoxicosis factitia?

ANSWER: The treatment of thyrotoxicosis factitia is usually psychiatric. It involves determining why patients resort to this trickery and convincing the patients of the need for more rational outlets for their emotions.

QUESTION: What is the full name and who is the producer of TSH?

ANSWER: Thyroid-stimulating hormone or thyrotropic hormone is not at present on the market. The Armour Laboratories and Parke-Davis have had it available for experimental purposes.

Answers by Charles W. Lloyd, Syracuse, N. Y.

QUESTION: Are there any reports in the literature of increased excretion of corticoids in premenstrual tension?

ANSWER: No definitive studies of the corticosteroid excretion by patients with premenstrual tension have been published. Venning and Kazmin (*Endocrinology* **39:** 131–139 (Aug.) 1946) detected no variation in glycogenic corticosteroid excretion which could be correlated with the normal menstrual cycle. Smith and Smith (*Recent Progr. Hormone Res.* **7:** 209–253, 1952) reported a premenstrual rise in formaldehydogenic corticosteroids on the basis of studies of a small number of cases. In our own laboratory a study of 12 normal women has not shown a significant rise in formaldehydogenic corticosteroid premenstrually, although a suggestion of a rise in 2 patients with premenstrual tension was observed. The number of cases is too few to be significant. Although corticosteroid excretion studies have not conclusively demonstrated a rise in adrenal cortical function premenstrually, the circulating eosinophil level falls at the time of ovulation and is lower during the luteal phase than before ovulation (Davis and Hulit: *J. Clin. Endocrinol.* **9:** 714–724 (Aug.) 1949). These authors did not study women with premenstrual tension. At the present time the available evidence does not prove the existence of altered adrenal cortical function in premenstrual tension, but the possibility of such a situation certainly exists.

QUESTION: Please discuss the use of estrogens in the treatment of adolescent acne and' the risks and disadvantages to the patient of such hormonal therapy.

ANSWER: In the young woman who has acne which becomes worse premenstrually estrogen therapy may be of considerable benefit. This is particularly so in the patient who has a grossly irregular cycle. In these patients cycle regulation, at first by rather large doses of estrogen (205 mg. of diethylstilbestrol) given in twenty-day courses, and subsequently by courses of small (0.1 mg. of diethylstilbestrol) daily doses designed to stimulate pituitary function, is often associated with considerable improvement in the acne. In the regularly cycling young woman it is sometimes justifiable to give rather large doses of estrogen in courses of twenty days, beginning on the fifth day of bleeding. This blocks ovulation and is sometimes associated with improvement of the acne. The only disadvantage of such treatment is the occasional production of an episode of functional bleeding, but this is unlikely to occur if the timing of therapy is carefully watched so that it coincides with the patient's own cycle. Our experience with local application of estrogen to the face has been disappointing.

In the male, our results with estrogen therapy have not been good. White and Lehmann (*Arch. Dermat. & Syph.* **65:** 601–608 (May) 1952) found no better response in 35 males treated with diethylstilbestrol, 1 to 2 mg. daily for twenty to seventy-seven days in addition to the usual dermatologic procedures, than in a comparable group of patients who did not receive the estrogen. Feminization and depression of testicular function are certainly undesirable disadvantages of this type of therapy. Because of these disadvantages and the uncertain results, systemic estrogen therapy in the male is not widely used.

QUESTION: What dose of estrogen should be used to stimulate LH?

ANSWER: Either diethylstilbestrol, 0.1 mg. daily, or ethinyl estradiol, 0.5 mg. daily, can be used effectively to stimulate the release of LH and probably of luteotropic hormone. These two substances appear to be the most effective of a number of estrogens tested.

The estrogens should be given beginning on the fifth day of the cycle and continued for from twelve to twenty days. In some subjects a better basal body temperature curve and endometrial response (improvement in histologic pattern and glycogen content) are obtained when the material is given for only twelve days, whereas in others a longer course of therapy seems to be more effective. This type of therapy should be given repeatedly; and in the woman who has had anovulatory ovarian failure for some time, it may be several months before ovulation occurs as a result of this treatment. Sometimes the addition of progesterone in the last five days of the twenty-day course is of value. It is important to stress that in the normal woman who has excellent corpus luteal function, the use of even these small doses of estrogen may disrupt normal pituitary-ovarian relationships, with the result that ovulation may be blocked; or, if ovulation does occur, endometrial response will not be as good as it was prior to the therapy.

QUESTION: What is the evidence that follicular cells produce estrogen? Granulosa rather than theca?

ANSWER: The sites of formation of estrogenic substances have been reviewed by Corner (*Physiol. Rev.* **18:** 154–172 (Apr.) 1938). The evidence for production of estrogen by the follicle can be summarized as follows: 1) in essentially all mammalia, estrus is associated with maturity of the follicles and the estrous cycle parallels the cycle of the follicles; 2) estrogen is present in follicular fluid in relatively large amounts; 3) ovarian stroma without follicles contains little or no estrogen, whereas follicle wall (mostly granulosa cells) contains large amounts of estrogen (Allen, E., *et al.*: *Am. J. Physiol.* **92:** 127, 1930); 4) Aschheim (*Med. Klin.* **22:** 2023, 1926) also found no estrogen in stroma without follicles but did not find estrogen in granulosa scraped from the wall of large follicles—rather he found estrogen in theca cells; 5) although destruction of follicles by x-ray treatment does not prevent estrogen formation (Parkes, A. S.; *Proc. Roy. Soc.* sB., **100:** 172, 1926; **102:** 51, 1927) the cells which persist are probably genetically and functionally similar to some of the usual epithelioid elements of the ovary; and 6) following a series of ingenious experiments involving ablation of the cortex of the ovary, Westman (*Arch. f. Gynäkol.* **158:** 476, 1934) concluded that theca cells are the seat of estrogen formation.

Evidence from ovarian tumors suggests that granulosa cells have estrogen-forming ability. Certainly granulosa-cell tumors, which are of a relatively "pure" type, are capable of secreting estrogen. Of course, it cannot be assumed that these granulosa cells are identical with those of the normal follicle; nevertheless, their close relationship to these cells is generally accepted. There is, therefore, evidence that both granulosa cells and theca cells can form estrogen.

Answers by Charles L. Buxton, New York, N. Y.

QUESTION: What is the value of ACTH and cortisone in the prevention of erythroblastosis fetalis? In toxemia of pregnancy?

ANSWER: ACTH and cortisone have been tried in attempts to alleviate or prevent toxemia of pregnancy without success. Therefore their use is not advised in this abnormality.

For an excellent summary and discussion of the present views concerning the value of ACTH and cortisone in the prevention of erythroblastosis fetalis the reader is referred to the "Obstetrical and Gynecological Survey" December 1952, Vol. 7, no. 6, pp. 777–784. The conclusion reached is that these substances are probably of no value in this disease.

QUESTION: Have corticoid levels been determined in toxemia of pregnancy?

ANSWER: An extensive review of the work done in connection with the above question has recently been published by J. C. Mastboom in *Gynaecologia* **34:** 4 (Oct.) 1952. There are further data in connection with this problem published by J. W. Jailer, in *Bull. Univ. California Med. Center* **2:** 803, 1951. The reports of various investigators are so contradictory that the questioner is referred to these publications, from which he can draw his own conclusions.

QUESTION: I have a patient in whom the typical Sheehan's syndrome developed following rupture of an ectopic pregnancy. Has a similar case been reported in the literature?

ANSWER: We do not happen to know of any case of acute postpartum pituitary necrosis which has occurred following an ectopic pregnancy but, considering the etiology of this disease, there is no reason why it should not occur in this instance.

QUESTION: If pregnancy is a physiologic Cushing's syndrome, how do you account for the pigmentation in pregnancy, since this is found in hypoadrenalism?

ANSWER: The statement that pregnancy is a physiologic Cushing's syndrome, I am sure would be questioned by many experts in pituitary-adrenal disease relationships. It is not generally believed that the pigmentation which occurs in pregnancy has any relation to the type of pigmentation which occurs in Addison's disease.

QUESTION: Please cite a bit of the evidence that the placenta may elaborate vitamins.

ANSWER: We can find no evidence that vitamins may be produced by the placenta.

QUESTION: Please comment on vitamin E and abortion.

ANSWER: There is no available adequate evidence that vitamin E is of any use in the treatment of abortion in the human.

QUESTION: Please discuss the increase in placental weight and improved vascularity of the placenta in patients receiving stilbestrol therapy. Why the increased fetal weight in stilbestrol-treated patient?

ANSWER: Smith and Smith (*Am. J. Obst. & Gynec.* **58:** 994, 1949) suggested an increase in placental weight in patients treated with stilbestrol during pregnancy. This finding has been confirmed by Sommers, Lawley and Hertig (*Am. J. Obst. & Gynec.* **58:** 1010, 1949). It is assumed that if increased fetal weight actually does occur in stilbestrol-treated patients it may possibly be due to increased placental circulation.

QUESTION: In what ways may pregnancy modify a glucose tolerance test?

ANSWER: Generally speaking, a glucose tolerance test during pregnancy may give a low initial value and a flatter curve than is ordinarily found.

QUESTION: Should labor be induced in every diabetic woman in pregnancy?

ANSWER: It is an old medical aphorism that the words "always" and "never" should never (*sic*) be used in connection with diagnosis and treatment. The implications of this question, however, are that the fetal mortality of diabetic mothers is so high, prenatal

antepartum death is so frequent, and the baby even in treated diabetics is likely to be so large, that greater fetal salvage might be effected by early initiation of labor. It is the general opinion of most reports on this subject that early initiation of labor is definitely advisable. Nevertheless, another medical aphorism may be quoted to the effect that "every case must be treated on its own merits" and factors such as the size of the baby, the inducibility of labor, and the condition of the patient's diabetes, must be carefully considered.

QUESTION: How do you treat or prevent side effects from large doses of Premarin, etc., recommended to stop bleeding and ovulation?

ANSWER: (By the term "Premarin" it is understood that the questioner means any estrogen. Actually Premarin is the trade name for a compound containing mostly estrone-sulfate and the question will be answered in terms of estrogens in general.) If one type and dosage of estrogen (for instance, diethylstilbestrol 5 mg. daily for twenty days) is used to prevent ovulation, but produces unpleasant side effects such as nausea and vomiting, another type of estrogen administered by mouth may be tried. It is suggested that either estrone-sulfate in amounts of about 5 mg. daily for the same length of time, or ethinyl-estradiol 1.5 mg. daily, be used instead. It is extremely rare to find any individual who will have unpleasant side effects from all these drugs, so that one which is appropriate to the individual patient can practically always be found. In occasional instances a persistence of administration of the estrogen in spite of the nausea and vomiting results in a cessation of these symptoms; and of course symptomatic treatment with Dramamine is certainly advisable.

QUESTION: Some investigators say that Premarin prevents ovulation; others say it stimulates ovulation. Which is correct?

ANSWER: (Here again it is assumed that the word "Premarin" means any estrogen and the question will be answered on the basis of this assumption.) There is no valid evidence that the administration of estrogen actually stimulates ovulation. If any estrogen is given to an individual in sufficient amounts it so depresses pituitary activity that gonadotropin secretion is greatly decreased and thus the ovary does not receive the appropriate stimulus to bring about ovulation; therefore, in this way, large dosages of estrogen may actually prevent ovulation. On the other hand, Zondek postulated many years ago that small continuous estrogen dosage stimulated pituitary activity and thus, indirectly, increased ovarian function. This is still believed by many to be the case; therefore, very small continuous estrogen dosage (in amounts of diethylstilbestrol up to no more than 0.3 mg. daily, or a corresponding amount in other estrogens) may possibly stimulate pituitary activity, whereas large doses of estrogen have exactly the opposite effect.

QUESTION: Have you seen climacteric symptoms occur years after the menopause? In other words, is it possible for a woman to have no symptoms with the natural menopause and years later begin to have hot flushes?

ANSWER: It is certainly possible to have hot flushes at any stage of later life but it cannot be considered that these symptoms are those of the menopause, since it and its concomitant estrogen withdrawal have occurred many years previously. There are many other causes for hot flushes, such as hypertensive cardiovascular disease, which should be investigated in the search for the etiology of the flushes.

QUESTION: Would high doses of stilbestrol (15 to 30 mg. per day) aggravate or produce hypertension?

ANSWER: There is no evidence available that large estrogen dosage might aggravate or produce hypertension.

Answers by Harry F. Klinefelter, Jr., Baltimore, Md.

QUESTION: Zondek states that small doses of estrogens stimulate the anterior pituitary. What is your opinion?

ANSWER: In both male and female rats, investigators generally agree that small doses of estrogens stimulate the anterior pituitary to release the luteinizing hormone (LH). There is some evidence to indicate that release of follicle-stimulating hormone (FSH) may also be slightly influenced. The mechanism of action is not understood.

In human females also, small doses of estrogens (0.1 mg. of stilbestrol daily) apparently stimulate the release of LH. This effect has been utilized in the treatment of delayed puberty and hypothalamic amenorrhea.

In human males, a similar effect has not been described but there is no reason to believe that it might not occur.

QUESTION: If ACTH contaminates intermedin (or vice versa) and influences pigmentation, how do you account for pigmentation in Addison's disease?

ANSWER: In Addison's disease, the pigmentation is caused by increased amounts of ACTH, which is being produced in excess of normal by the anterior pituitary. This would be expected, since one of the basic laws of endocrinology is at work: when the function of a target gland is sufficiently decreased or absent, the anterior pituitary produces an increased amount of the tropic hormone stimulating that gland.

QUESTION: What, exactly, is the Klinefelter syndrome?

ANSWER: This is a form of male hypogonadism which has also been termed "prepubertal seminiferous tubular failure."

The primary lesion is in the testis and becomes manifest at the time of puberty. The testes, which are descended, remain small—about 1.5 cm. in length. Histologic study shows extreme failure of development of the germinal epithelium, with hyalinization of tubules. Many Leydig cells are seen, often in clusters resembling adenomata, but the secretory activity of these cells is subnormal.

In most patients, there is enough androgen production to induce normal accessory sexual development. In some, however, there is definite eunuchoidism.

Most patients have gynecomastia, beginning at puberty, and all show azoospermia and sterility. There is invariably an increased urinary excretion of follicle-stimulating hormone (FSH)—more than 192 mouse units in twenty-four hours.

In summary, this syndrome is characterized by small testes, aspermatogenesis, relatively normal accessory sexual development, and increased urinary excretion of FSH. Gynecomastia is usually present. The etiology is unknown, and there is no known treatment for the primary testicular lesion. Substitution therapy with testosterone is sometimes indicated; the gynecomastia must be treated surgically.

QUESTION: Should x-ray treatment be given to a patient with malignant exophthalmos during or after estrogen-thyroid medication?

ANSWER: X-ray treatment for malignant exophthalmos should be given without regard to whether the patient has received or is receiving estrogen or thyroid or a combination of the two. It is presumed that the question refers to x-ray treatment to the pituitary gland, which is at present the treatment of choice.

Answers by Laurance W. Kinsell, Oakland, California

QUESTION: Can fat act as a reservoir for the storage of steroid hormone and thus prevent the onset of secondary sex characteristics?

ANSWER: To the best of my knowledge the answer to this question must be made largely by inference, insofar as the clinical aspects of the question are concerned.

There is no question that steroids are soluble in neutral fat. Hence it is entirely possible for steroids to be present in significant amount in adipose tissue. Two fairly common observations would suggest that steroids may be stored in fat tissue, and hence not be physiologically active. Fat boys very frequently have a delayed onset of puberty. In our experience, it appears that in some of them puberty may be precipitated by a weight reduction program, although this may be coincidental. Many fat women have relative or absolute amenorrhea. Irregular menses in such patients are frequently associated with lack of ovulation, as indicated by basal body temperature findings. Weight loss in response to suitable dietary instruction frequently results in the appearance of normal ovulatory cycles.

On the basis of the foregoing observations, the evidence seems rather good for the concept of storage of steroid hormones in body fat. It is recognized that other explanations may be invoked to explain these findings.

QUESTION: 1. Are there any peculiar or early electrocardiographic changes in acromegaly? 2. What is the cause of the heart failure?

ANSWER: 1. There are no specific or early changes recorded. A significant number of such patients have S-T depressions and intraventricular conduction defects.

2. The precise cause is unknown. Pathologically, one finds cardiac hypertrophy and interstitial fibrous tissue proliferation, both of which effects could be referable to the effect of the anterior pituitary growth hormone per se. Hypertension is not uncommonly present. There is also an increased work load associated with the increased total metabolism. This subject is discussed by Hejtmancik *et al.* (*Ann. Int. Med.* **34**: 1445 (June) 1951).

QUESTION: Have estrogens or androgens ever affected the diabetes of acromegaly favorably?

ANSWER: Dr. Perry McCullagh recently reported such a favorable effect. In a patient of ours currently under study and treatment, the insulin tolerance has become completely normal and the patient no longer has clinical diabetes. These findings are shown in the accompanying figure.

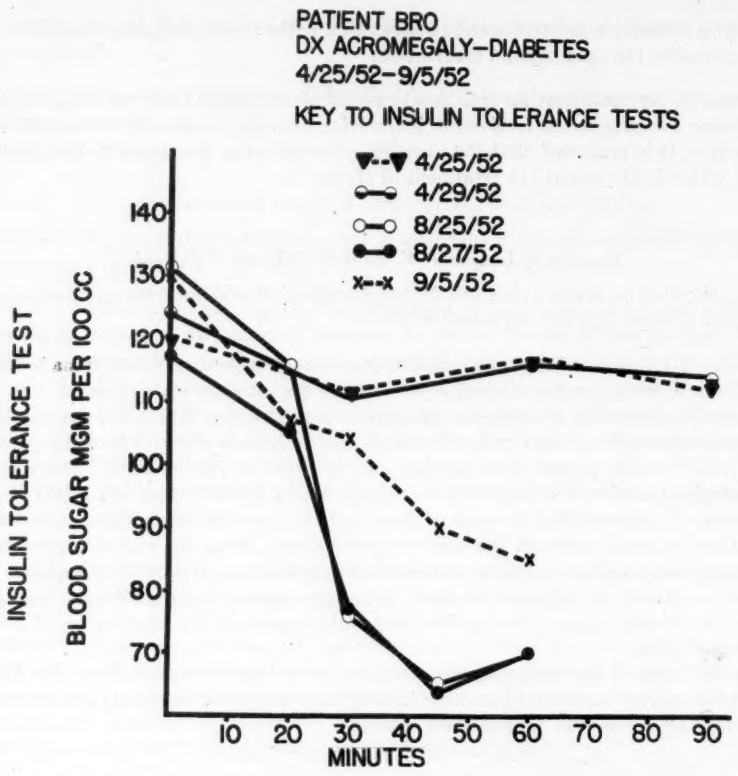

FIG. 1. Disappearance of insulin resistance in an acromegalic patient during prolonged estrogen therapy. The tests performed on April 25 and 29 were pre-estrogen; the remainder were during estrogen therapy.

QUESTION: Why are diabetes and hypermetabolism more common in acromegaly than in gigantism?

ANSWER: I am not certain that this is true. Gigantism occurs more rarely than acromegaly and consequently well controlled data are less available. In our own experience, 1 of 2 patients with gigantism had significant insulin resistance. "Hypermetabolism" is too broad a term to permit of evaluation.

QUESTION: Have you found any patients with acromegaly who are unable to tolerate estrogens because of edema?

ANSWER: No. The only problem which we have encountered is that which will occur in some of any significant number of female patients receiving large amounts of estrogen, namely, irregular excessive bleeding. With intermittent progesterone administration, this can usually be satisfactorily controlled.

QUESTION: Could acromegaly be caused by "temporary" hyperfunctioning of acidophils without the presence of adenoma?

ANSWER: One occasionally sees a patient with acromegaly or gigantism without a radiologically demonstrable pituitary adenoma. It is quite possible that pituitary hyperplasia, as the result of some hypothalamic pathology, could produce the symptom-picture of acromegaly.

The Endocrine Society

35TH ANNUAL MEETING

MAY 28–30, 1953

Headquarters: Hotel Statler, New York, New York.

Registration: Everyone attending the meetings is required to register. By action of the Council, non-members will be charged a registration fee of $5 for any part of the scientific sessions.

Fellows, graduate students, residents, and interns will be admitted upon presentation of proper credentials certified by a department head or superintendent of a hospital and payment of $2 registration fee.

Members should present their current membership cards when registering. Admission will be by badge only.

The Scientific Sessions: Scientific Sessions on Thursday, May 28, will be held in the Penn Top Room and on Friday and Saturday, May 29 and 30, in the Grand Ballroom of the Hotel Statler. (The Joint Session with the American Diabetes Association will be held in the Hotel Commodore, Saturday, May 30, at 2:00 P.M.) Sessions will begin promptly on schedule. Presentation of papers at all sessions will be LIMITED TO TEN MINUTES and authors are requested to adhere strictly to this schedule. Manuscripts of all papers should be submitted to the presiding officer or to the Secretary-Treasurer at the end of the presentation.

Annual Dinner: The Annual Dinner of the Society will be held on Friday evening, May 29, at 7:30 o'clock in the Georgian Room, preceded by cocktails at 6:30 P.M. in the same room. Secure tickets at time of registration.

Local Arrangements: The Committee on Local Arrangements for the meeting:—Dr. Earl T. Engle (Chairman), Dr. Rulon W. Rawson, Dr. Sydney C. Werner, and Dr. Edward Rall.

Secretary-Treasurer: Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma.

(For program, *see* April issue of the *Journal*)

THE AMERICAN GERIATRICS SOCIETY

The 10th Annual Meeting of the American Geriatrics Society will be held at the Hotel Commodore, New York City, May 28–30, 1953.

All members who plan to attend the meeting should reserve accommodations at the Hotel Commodore at an early date. The meeting is being held just before that of the American Medical Association and the hotels expect to be filled to capacity.

The program is probably the most interesting one that has been arranged for the Society up to the present time.

The Annual Dinner of the Society will be held on Friday evening, May 29, at 7:30 P.M., preceded by cocktails at 6:30 P.M. Wives and guests of members are welcome.

EUROPEAN SYMPOSIUM ON CORTISONE

During August 31 and September 1 and 2, 1953, a European Symposium on cortisone and the suprarenal cortex will be held in Milan by the University of Studies sponsored by the Società Italiana di Reumatologia. Scientists qualified in the subject have been invited to the meeting. The Symposium will deal with biologic and clinical topics, arranged as short presentations lasting not more than twenty minutes. A complete discussion, open to all persons, will follow each presentation. Italian, French and English are the official languages.

For information please apply to Prof. C. B. Ballabio, Secretary of the Congress, Clinica Medica dell'Università di Milano, via F. Sforza 35, Milano, Italy.